CHAPTER

1 Clinical Chemistry

contents

Note: *The reference ranges used throughout the book are meant to function as guides to understand and relate to the analytes; each laboratory facility will have established its own reference ranges based on the laboratory's specific instrumentation, methods, population, and so on.*

I. INSTRUMENTATION AND ANALYTICAL PRINCIPLES

A. Spectrophotometry General Information

1. **Electromagnetic radiation** has wave-like and particle-like properties.
   a. **Radiant energy** is characterized as a spectrum from short wavelength to long wavelength: cosmic, gamma rays, X-rays, ultraviolet, visible, infrared, microwaves, radiowaves.
   b. **Wavelength (λ)** is the distance traveled by one complete wave cycle (distance between two successive crests) measured in **nanometers (nm).**
   c. The **shorter the wavelength,** the **greater the energy** contained in the light, and the greater the number of photons.
   d. Light is classified according to its wavelength: **Ultraviolet (UV) light** has very short wavelengths and **infrared (IR) light** has very long wavelengths. When all **visible wavelengths** of light (400–700 nm) are combined, white light results.
      1) Visible color: wavelength of light transmitted (not absorbed) by an object
2. Particles of light are called **photons.** When an atom absorbs a photon, the atom becomes **excited** in one of three ways: An electron is moved to a higher energy level, the mode of the covalent bond vibration is changed, or the rotation around its covalent bonds is changed.
   a. When energy is absorbed as a photon, an **electron** is moved to a higher energy level where it is **unstable.**
      1) An excited electron is not stable and will **return to ground state.**
      2) An electron will **emit** energy in the form of **light** (radiant energy) of a characteristic wavelength.
      3) Absorption or emission of energy forms a **line spectrum** that is characteristic of a molecule and can help identify a molecule.

B. Spectrophotometer

1. In order to determine the concentration of a light-absorbing analyte in solution, a **spectrophotometer measures light transmitted** by that analyte in solution. Such an analyte may absorb, transmit, and reflect light to varying degrees, but always of a characteristic nature for the analyte.
2. **Components of a spectrophotometer**
   a. Power supply
   b. Light source
   c. Entrance slit
   d. Monochromator
   e. Exit slit
   f. Cuvet/sample cell
   g. Photodetector
   h. Readout device

3. The **light source** or **exciter lamp** produces an intense, reproducible, constant beam of light.
   a. Types of incandescent lamps
      1) **Tungsten:** Most common, used in visible and infrared regions
      2) **Deuterium:** Used in the ultraviolet region
   b. **Important:** When a lamp is changed in the spectrophotometer, the instrument must be recalibrated, because changing the light source changes the angle of the light striking the monochromator.
4. **Monochromators**
   a. **Glass filters** and **interference filters** are used in **photometers.**
   b. **Diffraction gratings** and **prisms** are used in **spectrophotometers.**
   c. The **bandpass** or **spectral bandwidth** is the range of wavelengths in nanometers that is transmitted by the monochromator and exit slit between two points of a spectral scan where the light transmitted is one-half of the peak (maximum) transmittance. This means if wavelengths 550 and 560 nm pass 50% of the maximum transmitted light, the range of wavelengths between 550 and 560 nm represents a 10-nm bandpass.
   d. **Wavelength selection:** Entrance slit allows lamp light to enter; slit is fixed in position and size. Monochromator disperses the light into wavelengths. Exit slit selects the bandpass of the monochromator that allows light of the selected wavelength to pass through the cuvet onto the detector.
5. **Photodetectors:** A **detector converts** the **electromagnetic radiation** (light energy) transmitted by a solution **into an electrical signal.** The more light transmitted, the more energy, and the greater the electrical signal that is measured.
6. **Readout devices:** Electrical energy from a detector is displayed on some type of digital display or readout system. The readout system may be a chart recorder or a computer printout.

C. Atomic Absorption Spectrophotometry

1. **Principle: Ground-state atoms absorb light at defined wavelengths.**
   a. **Line spectrum** refers to the wavelengths at which an atom absorbs light; each metal exhibits a specific line spectrum.
   b. The sample is **atomized in a flame** where the atoms of the metal to be quantified are maintained at ground state.
   c. Then a beam of light from a **hollow-cathode lamp** (HCL) is passed through a chopper to the flame.
   d. The **ground-state atoms** in the flame **absorb** the same wavelengths of **light** from the HCL as the atoms emit when excited.
   e. The **light not absorbed** by the atoms is **measured as a decrease in light intensity by the detector.** The detector (photomultiplier tube) will selectively read the pulsed light from the chopper that passes through the flame and will not detect any light emitted by the excited atoms when they return to ground state.

f. The difference in the amount of light leaving the HCL and the amount of light measured by the detector is **indirectly proportional to the concentration** of the metal analyte in the sample.

2. **Components**

Hollow-cathode lamp → chopper → burner head for flame → monochromator → detector → readout device

3. **Hollow-cathode lamp**
   a. HCL contains an anode, a cylindrical cathode made of metal being analyzed, and an inert gas such as helium or argon.
   b. **Principle:** Applied voltage causes ionization of the gas, and these excited ions are attracted to the cathode, where they collide with the metal coating on the cathode, knocking off atoms and causing atomic electrons to become excited. When the electrons of the metal atoms from the cathode return to ground state, the characteristic light energy of that metal is emitted.
   c. Vaporized metal atoms from the sample can be found in the flame. The flame serves as the sample cuvet in this instrument.
   d. The light produced in the HCL passes through a chopper and then to the flame, and the light is absorbed by the metal in the sample. The light not absorbed will be read by the photomultiplier tube.
   e. A **flameless system** employs a carbon rod (graphite furnace), tantalum, or platinum to hold the sample in a chamber. The temperature is raised to vaporize the sample being analyzed. The atomized sample then absorbs the light energy from the HCL. This technique is more sensitive than the flame method.

D. Nephelometry

1. **Definition:** Nephelometry is the **measurement of light scattered** by a particulate solution. Generally, scattered light is measured at an angle to the incident light when small particles are involved; for large molecules, forward light scatter can be measured. The **amount of scatter is directly proportional** to the number and size of particles present in the solution.
2. The **sensitivity of nephelometry** depends on the absence of background scatter from scratched cuvets and particulate matter in reagents.

E. Turbidimetry

1. **Definition:** Turbidimetry **measures light blocked** as a decrease in the light transmitted through the solution; dependent on particle size and concentration.
2. **Turbidimetry uses a spectrophotometer** for measurement, and it is limited by the photometric accuracy and sensitivity of the instrument.

F. Molecular Emission Spectroscopy

1. Types of **luminescence** where **excitation requires absorption of radiant energy**
   a. **Fluorescence** is a process where atoms absorb energy at a particular wavelength (excitation), electrons are raised to higher-energy orbitals, and

the electrons release energy as they return to ground state by emitting light energy of a longer wavelength and lower energy than the exciting wavelength. The emitted light has a very short lifetime.

1) **Fluorometry:** Frequently UV light is used for excitation and is passed through a primary filter for proper wavelength selection for the analyte being measured. The excitation light is absorbed by the atoms of the analyte in solution, which causes the electrons to move to higher-energy orbitals. Upon return to ground state, light is emitted from the fluorescing analyte and that light passes through a secondary filter. The secondary filter and the detector are placed at a right angle to the light source to prevent incident light from being measured by the detector. Whereas fluorometers use filters, spectrofluorometers use prisms or diffraction gratings as monochromators.
2) **Advantages:** Fluorometry is about 1000 times more **sensitive** than absorption techniques and has increased **specificity** because optimal wavelengths are chosen both for absorption (excitation) and for monitoring emitted fluorescence.
3) **Limitations:** Changes from the established protocol that affect pH, temperature, and solvent quality; self-absorption; quenching

b. **Phosphorescence** is the emission of light produced by certain substances after they absorb energy. It is similar to fluorescence except that the time delay is longer (greater than 10^{-4} sec) between absorption of radiant energy and release of energy as photons of light.

2. Types of **luminescence** where excitation does **not** require **absorption of radiant energy**
   a. **Chemiluminescence** is the process where the **chemical energy** of a reaction produces excited atoms, and upon electron return to ground state, photons of light are emitted.
   b. **Bioluminescence** is the process where an **enzyme-catalyzed** chemical reaction produces light emission. For example, this may occur in the presence of the enzyme luciferase because of oxidation of the substrate luciferin.
      1) **Luminometer** is a generic term for the type of instrument that is used to measure chemiluminescence and bioluminescence.

G. Chromatography

1. **Chromatography** is a technique where solutes in a sample are separated for identification based on **physical differences** that allow their differential distribution between a mobile phase and a stationary phase.
2. **Mobile phase:** May be an inert gas or a liquid
3. **Stationary phase:** May be silica gel bound to the surface of a glass plate or plastic sheet; may be silica or a polymer that is coated or bonded within a column

H. Thin-Layer Chromatography (TLC)

1. TLC is a type of planar chromatography. The **stationary phase** may be silica gel that is coated onto a solid surface such as a glass plate or plastic sheet. The

mobile phase is a solvent, where solvent polarity should be just enough to achieve clear separation of the solutes in the sample. TLC is a technique used clinically for **urine drug screening.**

2. **The mobile phase moves through the stationary phase by absorption and capillary action.** The solute components move at different rates because of solubility in the mobile phase and electrostatic forces of the stationary phase that retard solute movement. These two phases work together to provide **solute resolution and separation.**
   a. Solute will stay with the **solvent front** if solvent is too polar for the solute.
   b. Solute will remain at **origin** if solvent is insufficiently polar.
3. Basic steps in performing TLC include sample extraction using a liquid-liquid or column technique; concentration of the extracted sample; sample application by spotting onto the silica gel plate; development of the solute in the sample using the stationary and mobile phases; solute detection using chromogenic sprays, UV light, fluorescence, and heat; and interpretation of chromatographic results utilizing R_f values of solutes in comparison to aqueous standards.
4. **R_f values** are affected by chamber saturation, temperature, humidity, and composition of the solvent.

I. Gas-Liquid Chromatography (GLC)

1. **Gas-liquid chromatograph** components include a carrier gas with a flow-control device to regulate the gas flow, a heated injector, chromatographic column to separate the solutes, heated column oven, detector, and computer to process data and control the operation of the system.
2. **Gas-liquid chromatography** is a technique used to **separate volatile solutes.**
   a. The sample is injected into the injector component of the instrument where the **sample is vaporized** because the injector is maintained approximately 50°C higher than the column temperature.
   b. An **inert carrier gas (mobile phase)** carries the vaporized sample into the column. Carrier gases commonly used include hydrogen, helium, nitrogen, and argon. The **carrier gas flow rate is critical** to maintaining column efficiency and reproducibility of elution times.
   c. The types of **columns (stationary phase)** used are designated as packed or capillary. When the volatile solutes carried by the gas over the stationary phase of the column are eluted, the column effluent is introduced to the detector. The solutes are introduced to the detector in the order that each was eluted.
   d. The **detector** produces a signal for identification and quantification of the solutes. Commonly used detectors include flame ionization, thermal conductivity, electron capture, and mass spectrometer.
   e. Separation of solutes is a function of the relative differences between the vapor pressure of the solutes and the interactions of the solutes with the stationary column. The **more volatile** a solute, **the faster it will elute** from the column; the **less interaction** of the solute **with the column,** the **faster it will elute.**

f. **Identification** of a solute is based on its **retention time,** and **quantification** is based on **peak size,** where the amount of solute present is proportional to the size of the peak (area or height of the sample peak is compared to known standards).

J. High-Performance Liquid Chromatography (HPLC)

1. **High-performance liquid chromatograph** components include solvent reservoir(s), one or more pumps to propel the solvent(s), injector, chromatographic column, detector, and computer to process data and control the operation of the system.
2. HPLC is a type of liquid chromatography where the **mobile phase** is a **liquid** that is passed over the **stationary phase** of the **column.** The separation of solutes in a sample is governed by the selective distribution of the solutes between the mobile and stationary phases.
   a. **Solvents** commonly used for the **mobile phase** include acetonitrile, methanol, ethanol, isopropanol, and water.
      1) **Isocratic elution:** Strength of solvent **remains constant** during separation.
      2) **Gradient elution:** Strength of solvent **continually increases** (%/min) during separation.
   b. **Stationary phase** is an **organic material covalently bonded to silica** that may be polar or nonpolar in composition.
      1) **Normal-phase** liquid chromatography: Polar stationary phase and nonpolar mobile phase
      2) **Reversed-phase** liquid chromatography: Nonpolar stationary phase and polar mobile phase
3. The **solvent-delivery system** utilizes a solvent reservoir from which the pump can push the mobile phase through the column. The sample is introduced through a loop injector. A pre-column and guard column function to maintain the integrity of the column and are positioned prior to the sample reaching the main column. The column, which functions as the stationary phase, generally operates at room temperature. The effluent from the column passes to a detector system. The solutes are introduced to the detector in the order that each was eluted.
4. The **detector** produces a signal for identification and quantification of the solutes. Commonly used detectors include spectrophotometer, photodiode array, fluorometer, electrochemical, and mass spectrometer.

K. Mass Spectrometry

1. A **mass spectrometer** is an instrument that uses the principle of **charged particles moving through a magnetic or electric field,** with **ions** being **separated** from other charged particles **according to their mass-to-charge ratios.** In this system, electrons bombard a sample, ionizing the compound into **fragment ions,** which are separated by their mass-to-charge ratios. The **mass**

spectrum produced is unique for a compound **(identification),** and the **number of ions** produced relates proportionally to **concentration** (quantification).

2. **Mass spectrometry** is a high-quality technique for identifying drugs or drug metabolites, amino acid composition of proteins, and steroids. In addition, mass spectrometry has applications in the field of proteomics. The **eluate gas from a gas chromatograph** may be introduced into a mass spectrometer that functions as the detector system, or the **liquid eluate** may be introduced **from** a high-performance liquid chromatograph.
3. **Instrumentation**
   a. **Mass spectrometer** components include ion source, vacuum system, analyzer, detector, and computer.
   b. **Ion source:** Samples enter the ion source and are bombarded by the ionization beam. When the sample is in gas form and introduced from a gas chromatograph, the ion source may be electron or chemical ionization. Other types, such as electrospray ionization and sonic spray ionization, may be used when a high-performance liquid chromatograph is used in conjunction with a mass spectrometer.
   c. **Vacuum system:** Prevents the collision of ions with other molecules when electronic or magnetic separation is occurring
   d. Analyzer: Beam-type and trapping-type
      1) **Beam-type** is a destructive process, where ions pass through the analyzer one time and then strike the detector.
      2) **Quadrupole** is a beam-type analyzer, where mass-to-charge ratios are scanned during a prescribed time period to form a mass spectrum.
   e. **Detector** usually detects ions using electron multipliers, such as discrete dynode and continuous dynode electron multipliers.
   f. **Computer and software** convert the detector's signal to a digital form. Sample **identification** is achieved because each compound produces a **unique spectrum,** which is analyzed by a database for matching to a computerized reference library.
4. To further improve selectivity and sensitivity, a system known as **tandem mass spectrometers** can be employed, where a gas chromatograph or a high-performance liquid chromatograph is connected to **two** mass spectrometers (GC/MS/MS) or (HPLC/MS/MS). In these systems, ions of a specific mass-to-charge ratio are allowed to continue to the **second mass spectrometer,** where **additional fragmentation** occurs and final analysis is done.

L. Polarography

1. **Polarography** employs an **electrochemical cell.**
   a. Gradually increasing the voltage applied between two electrodes of the cell in contact with a solution containing the analyte
   b. Current measured; voltage change versus current plotted to produce a polarogram

   c. Voltage at which sharp rise in current occurs characteristic of the electrochemical reaction involved
   d. Amount of increase in current (i.e., the wave height) proportional to the concentration of analyte
2. **Anodic stripping voltammetry** is based on polarography.
   a. Negative potential applied to one of the electrodes
   b. Trace metal ions in the solution reduced and **plated onto anodic electrode;** preconcentrating step
   c. Plated electrode used as anode in polarographic cell; **metal stripped off anode**
   d. Current flow during stripping provides polarogram that **identifies** and **quantifies** the analyte being measured (trace metals)
   e. Used to assay heavy metals such as **lead in blood**

M. Potentiometry

1. **Potentiometry** is a technique used to determine the concentration of a substance in solution employing an **electrochemical cell** that consists of two half-cells, where the potential difference between an indicator electrode and a reference electrode is measured.
   a. Half-cell, also called an electrode, composed of single metallic conductor surrounded by solution of electrolyte
   b. Two different half-cells connected to make complete circuit; current flows because of potential difference between two electrodes
   c. Salt bridge connection between two metallic conductors and between two electrolyte solutions
   d. Comparison made between the voltage of one half-cell connected to another half-cell
   e. Half-cell potentials compared to potential generated by standard electrode
   f. Universally accepted standard half-cell is the standard hydrogen electrode, arbitrarily assigned a potential E° of 0.000 volt.
   g. Desirable to use one half-cell (reference electrode) with known and constant potential, not sensitive to composition of material to be analyzed
   h. Calomel electrode type of **reference electrode,** consisting of mercury covered by a layer of mercurous chloride in contact with saturated solution of potassium chloride
   i. Other half-cell **(indicator electrode)** selected on basis of change in its potential with change in concentration of analyte to be measured
   j. Silver-silver chloride (Ag/AgCl) electrode; common type of reference electrode
2. A **pH/blood gas analyzer** employs a pH-sensitive glass electrode for measuring blood pH, and it employs PCO_2 and PO_2 electrodes for measuring gases in blood. For measuring pH, the **pH electrode** is a functioning **glass electrode** that is dependent on properties of pH-sensitive glass.

a. Glass electrode made by sealing thin piece of pH-sensitive glass at the end of glass tubing and filling tube with solution of hydrochloric acid saturated with silver chloride
b. Silver wire immersed in tube's solution with one end extending outside the tube for external connection; silver-silver chloride reference electrode sealed within tube with pH-sensitive glass tip
c. pH-sensitive glass must be saturated with water. Surface of the glass develops a hydrated lattice, allowing exchange of alkaline metal ions in the lattice for hydrogen ions in the test solution. A potential is created between the inside and the outside of the electrode, and that potential is measured.
d. Glass electrode calibrated by comparison with two primary standard buffers of known pH
e. Because pH readings are temperature sensitive, the calibration must be carried out at a constant temperature of 37°C.

3. In a pH/blood gas analyzer, the ***PCO_2* electrode** for measuring the **partial pressure of carbon dioxide (*PCO_2*)** in blood is actually a pH electrode immersed in a bicarbonate solution.
   a. The bicarbonate solution is separated from the sample by a membrane that is permeable to gaseous CO_2 but not to ionized substances such as H^+ ions.
   b. When CO_2 from the sample diffuses across the membrane, it dissolves, forming carbonic acid and thus lowering the pH.
   c. The pH is inversely proportional to the log of the PCO_2. Hence, the scale of the meter can be calibrated directly in terms of PCO_2.
4. The **ion-exchange electrode** is a type of potentiometric, **ion-selective electrode.**
   a. Consists of liquid ion-exchange membrane made of inert solvent and ion-selective neutral carrier material
   b. Collodion membrane may be used to separate membrane solution from sample solution
   c. **K^+ analysis:** Antibiotic **valinomycin,** because of its ability to bind K^+, used as a neutral carrier for K^+-selective membrane
   d. **NH_4^+ analysis:** Antibiotics **nonactin** and **monactin** used in combination as neutral carrier for NH_4^+-selective membrane
5. **Sodium analysis:** Ion-selective electrodes based on principle of potentiometry
   a. Utilize **glass membrane electrodes** with selective capability
   b. Constructed from glass that consists of silicon dioxide, sodium oxide, and aluminum oxide

N. Amperometry: Electrochemical technique that measures the amount of current produced through the oxidation or reduction of the substance to be measured at an electrode held at a fixed potential

1. In a pH/blood gas analyzer, the electrode for measuring the **partial pressure of oxygen (*PO_2*)** in the blood is an electrochemical cell consisting of a platinum cathode and a Ag/AgCl anode connected to an external voltage source.

2. The cathode and anode are immersed in the buffer. A polypropylene membrane selectively permeable to gases separates the electrode and buffer from the blood sample.
3. When there is no oxygen diffusing into the buffer, there is practically no current flowing between the cathode and the anode because they are polarized.
4. When oxygen diffuses into the buffer from a sample, it is reduced at the platinum cathode.
5. The electrons necessary for this reduction are produced at the anode. Hence a current flows; the current is directly proportional to the PO_2 in the sample.

O. Coulometry

1. A **chloride coulometer** employs a coulometric system based on Faraday's law, which states that in an electrochemical system, the **number of equivalent weights of a reactant oxidized or reduced is directly proportional to the quantity of electricity used in the reaction.** The quantity of electricity is measured in coulombs. The coulomb is the unit of electrical quantity; 1 coulomb of electricity flowing per minute constitutes a current of 1 ampere.
2. If the current is constant, the number of equivalent weights of reactant oxidized or reduced **depends only on the duration of the current.**
3. In the chloride coulometer, the electrochemical reaction is the generation of Ag^+ ions by the passage of a direct current across a pair of silver electrodes immersed in a conducting solution containing the sample to be assayed for chloride. As the Ag^+ ions are generated, they are immediately removed from solution by combining with chloride to form insoluble silver chloride. When all the chloride is precipitated, **further generation of Ag^+ ions** causes an **increase in conductivity** of the solution.
4. The **endpoint** of the titration is indicated by the **increase in conductivity** of the solution. **Amperometry** is used to **measure the increase in conductivity.**

P. Electrophoresis

1. Used clinically to separate and identify proteins, including serum, urine and cerebrospinal fluid (CSF) proteins, lipoproteins, isoenzymes, and so on.
2. **Electrophoresis** is defined as the **movement of charged molecules** in a liquid medium when an electric field is applied.
3. **Zone electrophoresis** is defined as the movement of charged molecules in a porous supporting medium where the **molecules separate as distinct zones.**
4. **Support medium** provides a matrix that allows molecules to separate (e.g., agarose gel, starch gel, polyacrylamide gel, and cellulose acetate membranes).
5. Movement of charged particles through a medium depends on the nature of the particle, including net charge, size and shape, the character of the buffer and supporting medium, temperature, and the intensity of the electric field.
   a. Nature of the charged particle: **Proteins are amphoteric** and may be charged positively or negatively depending on the pH of the buffer solution.

   b. The pH at which negative and positive charges are equal on a protein is the **protein's isoelectric point.**
6. **Buffer solutions of pH 8.6** are generally used for serum protein electrophoresis. Using agarose gel or cellulose acetate at this alkaline pH, **serum proteins** take on a **net negative charge** and will migrate toward the **anode (+).** Albumin migrates the fastest toward the anode and the gamma-globulins remain closer to the cathode (–).
7. **Visualizing the separated analyte:** Following electrophoresis, treat the support medium with colorimetric stains or fluorescent chemicals. **Amido black B, Ponceau S, and Coomassie brilliant blue stains** are used for visualizing **serum proteins.** Silver nitrate is used for CSF proteins, fat red 7B and oil red O are used for lipoproteins, and nitrotetrazolium blue is used for lactate dehydrogenase isoenzymes.
8. Detection and quantification of the separated protein is accomplished using a **densitometer.**
9. Commonly encountered problems in electrophoresis
   a. **Holes in staining pattern:** Analyte present in too high a concentration
   b. **Very slow migration:** Voltage too low
   c. **Sample precipitates in support:** pH too high or low; excessive heat production
10. **Isoelectric focusing** is a type of zone electrophoresis in which protein separation is based on the **isoelectric point (pI)** of the proteins. This method utilizes polyacrylamide or agarose gel containg a pH gradient formed by ampholytes in the medium. When exposed to an electric field, the ampholytes migrate based on their pI to their respective positions in the gradient. In turn, the serum proteins will migrate in the gel to the position where the gel's pH equals the pI of the respective protein.
11. **Capillary electrophoresis** is based on **electroosmotic flow** (EOF). When an electric field is applied, the flow of liquid is in the direction of the cathode. Thus, EOF regulates the speed at which solutes move through the capillary.

Q. Hemoglobin Electrophoresis

1. **Hemoglobin:** Tetramer composed of **four globin chains,** four heme groups, and four iron atoms
   a. **Hemoglobin A_1:** Two alpha chains and **two beta chains**
   b. **Hemoglobin A_2:** Two alpha chains and **two delta chains**
   c. **Hemoglobin F:** Two alpha chains and **two gamma chains**
2. A number of hemoglobinopathies exist where a **substitution** of one amino acid on either the alpha chain or the beta chain causes the formation of an abnormal hemoglobin molecule.
   a. **Hemoglobin S:** Substitution of valine for glutamic acid in position 6 of the beta chain.
   b. **Hemoglobin C:** Substitution of lysine for glutamic acid in position 6 of the beta chain.

3. Although hemoglobin differentiation is best achieved by use of electrophoresis, hemoglobin F may be differentiated from the majority of human hemoglobins because of its alkali resistance.
4. At **pH 8.6,** hemoglobins have a net negative charge and migrate from the point of application toward the anode. Using **cellulose acetate:**
   a. Hemoglobin A_1 moves the fastest toward the anode, followed by hemoglobin F and hemoglobins S, G, and D, which migrate with the same mobility.
   b. Hemoglobins A_2, C, O, and E have the same electrophoretic mobility and migrate slightly slower than hemoglobin S, G, and D.
5. At **pH 6.2 on agar gel,** hemoglobins exhibit different electrophoretic mobilities in comparison with hemoglobins electrophoresed at pH 8.6 on cellulose acetate.
   a. Order of migration, from the most anodal hemoglobin to the most cathodal hemoglobin, is hemoglobins C and S; followed by hemoglobins A_1, A_2, D, E, and G, which migrate as a group with the same mobility; followed by hemoglobin F.
   b. The different migration patterns seen with cellulose acetate at pH 8.6 and agar gel at pH 6.2 are useful in differentiating hemoglobins that migrate with the same electrophoretic mobility.

R. Automation Parameters/Terminology

1. **Centrifugal analysis:** Centrifugal force moves samples and reagents into cuvet areas for simultaneous analysis.
2. **Discrete analysis:** Each sample reaction is compartmentalized. This may relate to an analyzer designed to assay only one analyte (e.g., glucose) or an analyzer capable of performing multiple tests where the sample and reagents are in a separate cuvet/reaction vessel for each test.
3. **Random access:** Able to perform individual tests or panels, and allows for stat samples to be added to the run ahead of other specimens
4. **Batch analysis:** Samples processed as a group
5. **Stand-alone:** Instrument from a single discipline with automated capability
6. **Automated stand-alone:** Instrument from a single discipline with additional internal automated capability (e.g., auto-repeat and auto-dilute)
7. **Modular workcell:** At least two instruments from a single discipline with one controller
8. **Multiple platform:** Instrument able to perform tests from at least two disciplines
9. **Integrated modular system:** At least two analytical modules supported by one sample and reagent processing and delivery system
10. **Pneumatic tube system:** Transports specimens quickly from one location to another
11. **Throughput:** Maximum number of tests generated per hour

12. **Turnaround:** Amount of time to generate one result
13. **Bar coding:** Mechanism for patient/sample identification; used for reagent identification by an instrument
14. **Dead volume:** Amount of serum that cannot be aspirated
15. **Carry-over:** The contamination of a sample by a previously aspirated sample
16. **Reflex testing:** Use of preliminary test results to determine if additional tests should be ordered or cancelled on a particular specimen; performed manually or automated
17. **Total laboratory automation:** Automated systems exist for laboratories where samples are received, centrifuged, distributed to particular instruments using a conveyor system, and loaded into the analyzer without operator assistance. This kind of automation is seen in large medical center laboratories and commercial laboratories where the volume of testing is high.

S. Principles of Automation

1. Automated instruments use robotics and fluidics to replicate manual tasks.
2. **Specimen handling:** Some instruments have **level-sensing probes** that detect the amount of serum or plasma in the tube. Some systems have a reading device that **allows bar-coded sample tubes** to be loaded onto the instrument. Although not as common, other instruments require the operator to **manually enter** the position of the patient sample.
3. **Reagents**
   a. **Dry reagents** can be packaged as **lyophilized powder** or **tablet form** that must be reconstituted with a buffer or reagent-grade water. Reconstituting of reagents may need to be done manually and then the reagents placed on an analyzer for use, or reconstituting the reagents may be part of the total automation process as employed by the Dimension® analyzer.
   b. **Dry reagents** can be spread over a support material and assembled into a **single-use slide.** This technique is employed by the Vitros® analyzer.
   c. **Liquid reagents** are pipetted by the instrument and mixed with the sample.
4. **Testing Phase**
   a. Mixing of sample and reagents occurs in a vessel called a **cuvet.** Some instruments have permanent, nondisposable cuvets made of quartz glass. Other cuvets are made of plastic and are disposable.
   b. **Reaction temperatures and times** vary for each analyte. The most common reaction temperatures are 37°C and 30°C.
   c. **Kinetic assays:** Determination of sample concentration is based on **change in absorbance** over time.
   d. **Endpoint/colorimetric assays:** Incubated for a specific time, absorbance determined, absorbance related to calibrators for calculation of sample concentration
   e. A spectrophotometer is built within the system to read absorbances for kinetic and colorimetric assays. These systems may use a diffraction

grating or a series of high-quality filters. Some automated analyzers incorporate fluorometry or nephelometry.

5. **Data Management**
   a. The **computer module** of most automated instruments has a data management system that allows analysis of quality control (QC) materials and assessment of patient values (e.g., delta check) before releasing patient results.
   b. Instruments/laboratory information systems (LISs) also archive patient results and QC values. These archived results are stored by the laboratory for various lengths of time.

T. Point-of-Care Testing (POCT)

1. **Definition:** Performing diagnostic tests outside the main laboratory and at or near patient care areas
2. **Applications:** POCT is designed to provide immediate laboratory test results for immediate patient assessment and determination of appropriate treatment. POCT may be used in neonatal intensive care, coronary care, intensive care, or the emergency department.
3. **Operators:** Only waived laboratory tests can be performed using point-of-care instruments. Clinical laboratory technicians and clinical laboratory scientists must operate instruments that perform complex or high-complexity laboratory tests.
4. **Point-of-care (POC) instrument evaluations:** All POC instruments must be evaluated in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). The values obtained from POC instruments must correlate with values obtained from larger laboratory instruments. Linearity testing, calculation of control ranges, correlations of sample data, and reference ranges must be done for each instrument.
5. **Training:** All POC instrument operators must be trained, and training must be documented.
6. **Quality control:** All effective quality control systems must be set up for each POC instrument. The program must use appropriate standards and controls, statistical analyses, and a proficiency testing system. This information must be documented.

U. Immunochemical Techniques

1. **Immunoassays** encompass a number of immunochemical techniques used to detect an extremely small amount of analyte (functions as antigen) by reacting it with an antibody (functions as reagent) to form an **antigen-antibody complex.** The signal measured has a relationship to the **label** used, and the label may be attached to either a reagent antigen or a reagent antibody.
   a. **Detection limits:** Immunochemical techniques detect very small amounts of substances. Monoclonal antibodies increase the specificity of the procedure.

b. **Polyclonal antiserum:** Antibodies produced in an animal from many cell clones in response to an immunogen; heterogeneous mixture of antibodies
c. **Monoclonal antiserum:** Antibodies produced from a single clone or plasma cell line; homogeneous antibodies
d. **Used to quantify:** Hormones, tumor markers, drugs, and other analytes present in small concentrations

2. **Methods**
   a. **Competitive-binding immunoassays** are based on the **competition between an unlabeled antigen** (sample analyte) and a **labeled antigen** for an antibody. In this type of assay, the unlabeled antigen (sample analyte) is an unknown concentration and varies from sample to sample, whereas the labeled antigen concentration and the antibody concentration are constant for a particular method.
      1) As the assay proceeds, there will be some free labeled antigen remaining that does not bind to antibody.
      2) The concentration of the antibody binding sites is limited with respect to total antigens (unlabeled and labeled) present, which leads to less-labeled antigen bound to antibody when sample analyte concentration is high.
      3) It is then necessary to measure either the free labeled antigen or the labeled antigen-antibody complex and relate it to the concentration of analyte in the sample. Depending on the method, it may be necessary to separate the free labeled antigen from the labeled antigen-antibody complex.
         a) **Heterogeneous** assays require that free labeled antigen be physically removed from the labeled antigen bound to antibody. Radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and immunoradiometric assay (IRMA) are examples of this technique.
         b) **Homogeneous** assays do **not** require physical removal of free labeled antigen from bound-labeled antigen.
      4) The **original labels** used for immunoassays were **radioactive isotopes** (e.g., I^{125}); thus the term **radioimmunoassay.** Most immunoassays in use today use nonradioactive labels. **Enzyme** (e.g., alkaline phosphatase), **fluorophore** (e.g., fluorescein), and **chemiluminescent** (e.g., acridinium ester) **labels** are commonly used for immunoassays.
   b. **Enzyme multiplied immunoassay technique (EMIT)** is a **homogeneous** immunoassay where the **sample** analyte (functions as **unlabeled antigen**) competes with the **enzyme-labeled antigen** for the binding sites on the antibody. The more analyte (unlabeled antigen) present in the mixture, the less binding of enzyme-labeled antigen to the antibody. The unbound enzyme-labeled antigen will react with substrate because the enzyme is in

a conformational arrangement that allows for substrate to bind at the active site of the enzyme. The product formed is read spectrophotometrically. The more product formed, the greater was the concentration of analyte in the sample.

c. **Fluorescent polarization immunoassay (FPIA)** is based on measuring the degree to which fluorescence intensity is greater in one plane than in another **(polarized versus depolarized).** FPIA is based on the amount of polarized fluorescent light detected when the fluorophore label is excited with polarized light.
   1) FPIA is a **homogeneous** technique where the **sample** analyte (functions as **unlabeled antigen**) competes with the **fluorophore-labeled antigen** for the binding sites on the antibody. The more analyte (unlabeled antigen) present in the mixture, the less binding of fluorophore-labeled antigen to the antibody.
   2) The **free fluorophore-labeled antigen** has **rapid** rotation and emits **depolarized light.** The **fluorophore-labeled antigen-antibody complex** rotates more **slowly;** light is in the vertical plane **(polarized light)** and is detected as **fluorescence polarization.**
   3) The greater the concentration of analyte in the sample, the less binding between antibody and fluorophore-labeled antigen (bound complex emits polarized light), the greater the amount of free fluorophore-labeled antigen (emits depolarized light), and thus the lesser amount of polarization sensed by the detector. The amount of **analyte** in the sample is **inversely proportional** to the amount of fluorescence polarization. That is, the greater the concentration of analyte, the less the amount of polarized light detected.
   4) Used to measure hormones, drugs, and fetal pulmonary surfactant to assess fetal lung maturity

d. **Chemiluminescent immunoassay** is a technique between antigen and antibody that employs a chemiluminescent indicator molecule such as isoluminol and acridinium ester as labels for antibodies and haptens. In the presence of hydrogen peroxide and a catalyst, isoluminol is oxidized, producing light emission at 425 nm. In such an assay, the chemiluminescent signal is proportional to the concentration of analyte in the serum sample.

e. **Luminescent oxygen channeling immunoassay (LOCI™)** is a homogeneous technique that is an adaptation of the chemiluminescent immunoassay.
   1) Antigen (from serum sample) links to two antibody-coated particles. The first is an antibody-coated sensitizer particle containing a photosensitive dye (singlet oxygen source), and the second is an antibody-coated particle (singlet oxygen receptor) containing a precursor chemiluminescent compound and a fluorophore.

2) Irradiation of the immunocomplex produces singlet oxygen at the surface of the sensitizer particle that diffuses to the second particle being held in close proximity.
3) Singlet oxygen reacts with the precursor chemiluminescent compound to form a chemiluminescent product that decays and emits light. This light energy is accepted by a fluorophore, which results in light emission of a longer wavelength.
4) In this assay, the chemiluminescent signal is enhanced by the resulting fluorescent signal, which is proportional to the concentration of analyte in the serum sample.

f. **Electrochemiluminescence immunoassay** uses an indicator label such as **ruthenium** in sandwich and competitive immunoassays. Following a wash procedure to remove unbound label, label bound to magnetic beads at an electrode surface undergoes an electrochemiluminescent reaction with the resulting light emission measured by a photomultiplier tube.

II. PROTEINS AND TUMOR MARKERS

A. Characteristics of Proteins

1. Proteins are macromolecules made of **amino acids,** with each amino acid being linked to another via a **peptide bond.**
   a. **Peptide bond** is formed when the **carboxyl** ($-COOH$) group of one amino acid links to the **amino** ($-NH_2$) group of another amino acid with the loss of a water molecule.
   b. **N-terminal:** End of protein structure with a free amino group
   c. **C-terminal:** End of protein structure with a free carboxyl group
   d. **Nitrogen content:** Proteins consist of 16% nitrogen, which differentiates proteins from carbohydrates and lipids.
2. Protein structure
   a. **Primary structure:** The amino acids are linked to each other through covalent peptide bonding in a specific sequence to form a polypeptide chain.
   b. **Secondary structure:** The polypeptide chain winds to form alpha helixes and beta sheets through the formation of hydrogen bonds between CO and NH groups of the peptide bonds.
   c. **Tertiary structure:** The coiled polypeptide chain folds upon itself to form a three-dimensional structure through the interactions of the R groups of the amino acids. Such interactions include the formation of disulfide linkages, hydrogen bonds, hydrophobic interactions, and van der Waals forces.
   d. **Quaternary structure:** Two or more folded polypeptide chains bind to each other through hydrogen bonds and electrostatic interactions to form a functional protein.

B. Classification of Proteins

1. **Simple proteins:** Polypeptides composed of only amino acids
   a. **Globular proteins:** Symmetrical, compactly folded polypeptide chains (e.g., albumin)
   b. **Fibrous proteins:** Elongated, asymmetrical polypeptide chains (e.g., troponin and collagen)
2. **Conjugated proteins:** Composed of protein (apoprotein) and nonprotein (prosthetic group) components; **prosthetic groups** are commonly metal, lipid, and carbohydrate in nature
   a. **Metalloproteins:** Protein with a metal prosthetic group (e.g., ceruloplasmin)
   b. **Lipoproteins:** Protein with a lipid prosthetic group (e.g., cholesterol, triglyceride)
   c. **Glycoproteins:** Protein with 10–40% carbohydrates attached (e.g., haptoglobin)
   d. **Mucoproteins:** Protein with >40% carbohydrates attached (e.g., mucin)
   e. **Nucleoproteins:** Protein with DNA or RNA nucleic acids attached (e.g., chromatin)

C. Protein Functions

1. **Energy production:** Proteins can be broken down into amino acids that can be used in the citric acid cycle to produce energy.
2. **Water distribution:** Maintain the colloidal osmotic pressure between different body compartments
3. **Buffer:** The ionizable R groups of the individual amino acids of a protein provide buffering capacity by binding or releasing H^+ ions as needed.
4. **Transporter:** Binding of proteins to hormones, free hemoglobin, lipids, drugs, calcium, unconjugated bilirubin, and so on, allows movement of these and other molecules in the circulation.
5. **Antibodies:** Proteins that protect the body against "foreign" invaders
6. **Cellular proteins:** Function as receptors for hormones so that the hormonal message can activate cellular components; some hormones are protein in nature [e.g., adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH)]
7. **Structural proteins:** Collagen is the fibrous component that maintains the structure of body parts such as skin, bone, cartilage, and blood vessels.
8. **Enzymes:** Catalysts that accelerate chemical reactions

D. Plasma Total Protein

1. **Regulation**
   a. The **liver synthesizes** most of the **plasma proteins. Plasma cells synthesize** the **immunoglobulins.**

1) Proteins are synthesized from amino acids, with one amino acid linked to another through the formation of a peptide bond.
2) When proteins **degrade,** their constituent amino acids undergo **deamination** with the **formation of ammonia,** which is **converted to urea** for excretion in the urine.

b. Some **cytokines** released at the site of injury or inflammation cause the **liver** to **increase synthesis** of the **acute-phase reactant proteins.** This is a nonspecific response to inflammation that may be caused by autoimmune disorders or infections, as well as a nonspecific response to tissue injury from tumors, myocardial infarctions, trauma, or surgical procedures. On the other hand, some proteins will decrease in concentration and are referred to as **negative acute-phase proteins,** including prealbumin (transthyretin), albumin, and transferrin.

c. Immunoglobulins are humoral antibodies produced in response to foreign antigens for the purpose of destroying them.

d. **Reference ranges:** Total protein 6.5–8.3 g/dL; albumin 3.5–5.0 g/dL

2. In general, changes in total protein concentration are associated with:
   a. **Hypoproteinemia** caused by urinary loss, gastrointestinal tract inflammation, liver disorders, malnutrition, inherited immunodeficiency disorders, and extensive burns
   b. **Hyperproteinemia** caused by dehydration, increased protein production associated with monoclonal and polyclonal gammopathies, and chronic inflammatory diseases associated with paraprotein production

E. Clinical Significance of the Major Proteins

1. **Prealbumin** (also termed transthyretin): Indicator of nutritional status and is one of the proteins that transports thyroid hormones
   a. **Decreased** in liver disorders, inflammation, malignancy, and poor nutrition
   b. **Increased** in steroid therapy, chronic renal failure, and alcoholism
2. **Albumin** is synthesized in the liver and has the **highest concentration of all plasma proteins.** Albumin **binds** many analytes **for transport** in blood, including unconjugated bilirubin, steroids, ions such as calcium and magnesium, fatty acids, and drugs, and it significantly contributes to plasma **osmotic pressure.**
   a. **Decreased** in liver disorders because of decreased production, gastrointestinal disease associated with malabsorption, muscle-wasting diseases, severe burns caused by loss, renal disease caused by loss (nephrotic syndrome, glomerulonephritis), starvation, and malnutrition
   b. **Increased** in dehydration (relative increase)
3. **α_1-Antitrypsin** is an acute-phase reactant and a protease inhibitor that neutralizes trypsin-type enzymes that can damage structural proteins.
   a. **Decreased** in emphysema-associated pulmonary disease and severe juvenile hepatic disorders that may result in cirrhosis

b. **Increased** in inflammatory disorders

4. **α_1-Fetoprotein (AFP)** is synthesized during gestation in the yolk sac and liver of the fetus, peaking at 13 weeks and declining at 34 weeks. Normally, adult levels are very low.
   a. **Maternal serum AFP** is measured between 15 and 20 weeks of gestation and is reported as **multiples of the median** (MoM).
      1) **Increased** AFP level in maternal serum: neural tube defects, spina bifida, and fetal distress
      2) **Decreased** AFP level in maternal serum: Down syndrome, trisomy 18
   b. **In adults, increased** levels of AFP can be indicative of hepatocellular carcinoma and gonadal tumors.
5. **α_1-Acid glycoprotein (orosomucoid):** Acute-phase reactant; binds to basic drugs
   a. **Increased** in inflammatory disorders such as rheumatoid arthritis, pneumonia, and conditions associated with cell proliferation
   b. **Decreased** in nephrotic syndrome
6. **Haptoglobin:** α_2-globulin that binds free hemoglobin and is an acute-phase reactant
   a. **Increased** in inflammatory conditions, burns, trauma
   b. **Decreased** in intravascular hemolysis because of formation of a haptoglobin-hemoglobin complex for removal by the liver
7. **Ceruloplasmin** is an acute-phase reactant that is an α_2-globulin, **copper-containing protein** with enzymatic activity. Approximately 90% of serum copper is bound in ceruloplasmin.
   a. **Increased** in pregnancy, inflammatory disorders, malignancies, and with intake of oral estrogen and oral contraceptives
   b. **Decreased** in **Wilson disease,** malnutrition, malabsorption, severe liver disease
8. **α_2-Macroglobulin:** Proteolytic enzyme inhibitor that inhibits thrombin, trypsin, and pepsin
   a. **Increased** in nephrotic syndrome, contraceptive use, pregnancy, estrogen therapy
   b. **Decreased** slightly in acute inflammatory disorders and prostatic cancer; decreased markedly in acute pancreatitis
9. **Transferrin:** β-globulin that **transports iron**
   a. **Decreased** in infections, liver disease, and nephrotic syndrome
   b. **Increased** in iron-deficiency anemia and pregnancy
10. **C-reactive protein (CRP):** β-globulin that is an acute-phase reactant
    a. Increased in tissue necrosis, rheumatic fever, infections, myocardial infarction, rheumatoid arthritis, and gout
11. **Immunoglobulins:** Antibodies
    a. Five major classes: IgA, IgD, IgE, IgG, and IgM
       1) Synthesized in **plasma cells** as an immune response

2) One of the immunoglobulins will be increased in a **monoclonal gammopathy** (e.g., **multiple myeloma**). Such disorders are generally associated with an increase in IgG, IgA, or IgM; seldom is the increase associated with IgD or IgE.

b. **IgG** can cross the placenta.
   1) **Increased** in liver disorders, infections, and collagen disease
   2) **Decreased** in the presence of increased susceptibility to infection and when a monoclonal gammopathy is associated with an increase in another immunoglobulin

c. **IgA** levels increase after birth.
   1) **Increased** in liver disorders, infections, and autoimmune diseases
   2) **Decreased** in inhibited protein synthesis and hereditary immune disorders

d. **IgM** cannot cross the placenta; it is made by the fetus.
   1) **Increased** in various bacterial, viral, and fungal infections and **Waldenström macroglobulinemia**
   2) **Decreased** in renal diseases associated with protein loss and immunodeficiency disorders

e. **IgD** is increased in liver disorders, infections, and connective tissue disorders.

f. **IgE** is increased in allergies, asthma, and hay fever, and during parasitic infections.

12. **Fibronectin:** Fetal fibronectin is used to **predict risk of premature birth.** It is a normal constituent in the placenta and amniotic fluid. When stress, infection, or hemorrhage causes leakage of fibronectin into the cervicovaginal secretions, increased fibronectin is suggestive of risk for premature birth.

F. Methodology for Serum Total Protein, Albumin, and Protein Fractionation

1. **Refractometry** is based on the change in velocity of light (light is bent) as light passes through the boundary between air and water, which function as two transparent layers. In protein analysis, the light is bent and such change is proportional to the concentration of the solutes (proteins) present in the water (serum).
2. The **biuret method** is based on cupric ions complexing with peptide bonds in an alkaline medium to produce a purple-colored complex. The amount of purple complex produced is directly proportional to the number of peptide bonds present and reflects protein concentration.
3. **Dye binding** techniques allow proteins to bind to a dye, forming a protein-dye complex that results in a shift of the maximum absorbance of the dye (e.g., **Coomassie brilliant blue**). The increase in absorbance is directly proportional to protein concentration.
4. The **Kjeldahl** technique for the determination of total protein is too cumbersome for use in routine testing. It is considered the **reference method**

of choice to validate materials used with the biuret method. The Kjeldahl technique is based on the quantification of the nitrogen content of protein.

5. **Electrophoresis**
   a. **Serum protein electrophoresis:** Serum is applied in the cathode region of an agarose gel or cellulose acetate plate saturated with a buffer of pH 8.6. Serum proteins have a **net negative charge** and migrate toward the **anode,** with **albumin traveling the farthest,** followed by α_1-globulins, α_2-globulins, β-globulins, and γ-globulins. The proteins are fixed in the medium, stained, and then quantified using a densitometer.
   b. **High-resolution protein electrophoresis** is a modified technique that uses agarose gel, a higher voltage, a cooling system, and a more concentrated buffer to separate proteins into as many as 12 zones.
   c. **Isoelectric focusing** is a type of zone electrophoresis in which protein separation is based on the **isoelectric point (pI)** of the proteins.
6. **Immunochemical methods**
   a. Homogeneous and heterogeneous immunoassays
   b. Immunonephelometry
   c. Immunoelectrophoresis
   d. Radial immunodiffusion (RID)
   e. Electroimmunodiffusion
   f. Immunofixation
7. **Test methodology for albumin: Dye binding** techniques using **bromcresol green** and **bromcresol purple** dyes allow albumin to be positively charged for binding to the anionic dye, forming an albumin-dye complex that results in a shift of the maximum absorbance of the dye. The increase in absorbance is directly proportional to the albumin concentration.
8. **Test methodology for globulins:** The direct measurement of total globulins is not generally performed. The concentration of the globulins is determined by calculation. **Globulins = Total Protein − Albumin**

G. Proteins in Other Body Fluids

1. **Urinary proteins: Quantification** performed on 24-hour urine specimens
   a. **Test methods:** Sulfosalicylic acid, trichloroacetic acid, benzethonium chloride (turbidimetric), and Coomassie brilliant blue (spectrophotometric)
   b. **Reference range urine total protein:** 1–14 mg/dL; <100 mg/day
   c. **Clinical significance of proteinuria**
      1) **Increased** protein in urine may result from tubular or glomerular dysfunction, multiple myeloma, Waldenström macroglobulinemia, nephrotic syndrome
      2) **Bence Jones protein** may be found in urine of patients with multiple myeloma.
      3) Glomerular membrane can be damaged in diabetes, amyloidosis, and collagen diseases.

4) Glomerular dysfunction can be detected in its early stages by measuring microalbumin in urine. **Microalbuminuria** is a condition where the quantity of albumin in the urine is greater than normal, yet it is not able to be detected by the urine dipstick method. The presence of microalbuminuria in a diabetic individual is a concern because it **generally precedes nephropathy.**
   a) **Methods** for quantification: Enzyme immunoassays and immunonephelometric assays
   b) **Reference range for urine albumin:** <30 mg/day

2. **Cerebrospinal fluid (CSF) proteins**
   a. CSF is an **ultrafiltrate of plasma** formed in the ventricles of the brain.
   b. **Test methods** include sulfosalicylic acid, trichloroacetic acid, benzethonium chloride, and Coomassie brilliant blue.
   c. **Reference range:** 15–45 mg/dL
   d. **Clinical significance**
      1) **Increased** in viral, bacterial, and fungal meningitis, traumatic tap (bloody), multiple sclerosis, herniated disk, and cerebral infarction
      2) **Decreased** in hyperthyroidism and with central nervous system leakage of CSF

H. Tumor Marker Utilization

1. In general, tumor markers used today are **not very useful in diagnosis,** but they are **useful** in tumor staging, monitoring therapeutic responses, predicting patient outcomes, and detecting cancer recurrence. **Ideal characteristics** for tumor markers include:
   a. Measured easily
   b. High analytical sensitivity of assay method
   c. High analytical specificity of assay method
   d. Cost-effective
   e. Test results contribute to patient care and outcome
2. **Prostate specific antigen (PSA)**
   a. **Function**
      1) Produced by epithelial cells of the **prostate gland** and secreted into seminal plasma
      2) Glycoprotein protease that functions in liquefaction of seminal coagulum
   b. **Forms of PSA found in blood**
      1) **Enveloped** by protease inhibitor, α_2-macroglobulin; **lacks** immunoreactivity
      2) **Complexed** to another protease inhibitor, α_1-antichymotrypsin; **immunologically detectable**
      3) **Free PSA,** not complexed to protease inhibitor; **immunologically detectable**

4) **Total PSA** assays measure complexed and free PSA forms, as they are immunologically detectable.

c. **Specificity**
   1) PSA is a tissue-specific marker but **not** tumor specific.
   2) Small amounts present in serum normally
   3) Lacks specificity because serum level of PSA is increased in benign prostate hypertrophy as well as in adenocarcinoma of the prostate

d. **Prostate cancer detection**
   1) Early detection guidelines endorse lower cutoff of **PSA up to 2.5 ng/mL.**
   2) PSA>2.5 ng/mL perform biopsy
   3) **PSA velocity** is measurement of the **rate of change per year.**
      a) Biopsy recommended when PSA rises more than 0.75 ng/mL/year even when PSA is <2.5 ng/mL.
   4) **Free PSA:** Men with **prostate cancer** tend to have **lower % free PSA** (free PSA/total PSA) than men with benign disease. Lower % free PSA is associated with a higher risk of prostate cancer.
   5) PSA is used to monitor therapeutic response and to follow radical prostatectomy.

e. Methods used to measure serum levels of PSA include fluorescence immunoassay, enzyme immunoassay, and chemiluminescence immunoassay.

3. **α_1-Fetoprotein (AFP)**

a. Oncofetal glycoprotein antigen
   1) Synthesized in liver, yolk sac, and gastrointestinal (GI) tract of **fetus**
   2) Fetal serum AFP peaks at 12–15 weeks of gestation with levels of 2–3 mg/mL.
   3) At birth, levels fall to 50 μg/mL, and at 2 years of age only trace amounts are present.
   4) **Adult levels <20 ng/mL**

b. **Clinical significance**
   1) **Increased** levels of AFP in adults are associated with hepatocellular carcinoma, testicular and ovarian teratocarcinomas, pancreatic carcinoma, gastric and colonic carcinomas.
   2) **Increased** levels in adults are also seen in **nonmalignant** disorders, including viral hepatitis and chronic active hepatitis.
   3) Useful in monitoring therapeutic response of cancer patients to treatment protocols
   4) In **pregnancy, increased** maternal serum levels are associated with **spina bifida, neural tube defects,** and **fetal distress. Decreased** levels of maternal serum AFP are associated with increased incidence of **Down syndrome.**

c. Enzyme immunoassay methods are used for measurement.

4. **Carcinoembryonic antigen (CEA)**
   a. Oncofetal glycoprotein antigen
   b. Normally found in epithelial cells of the **fetal** GI tract
   c. **Clinical significance in adults**
      1) **Increased** levels of CEA are associated with adenocarcinoma of digestive tract and colorectal carcinoma.
      2) Elevations are seen in other malignancies and noncancerous disorders.
      3) Useful in monitoring therapeutic response of cancer patients to treatment protocols
   d. Enzyme immunoassay methods are used for measurement.
5. **Human chorionic gonadotropin (hCG)**
   a. hCG is a glycoprotein composed of α- and β-subunits. The **β-subunit is unique** and not common to other hormones; α-subunit is common to other hormones.
   b. Normally secreted by the trophoblast cells of the placenta
   c. **Increased** secretion is associated with **trophoblastic tumors,** choriocarcinoma, nonseminomatous testicular tumors, and ovarian tumors.
   d. Useful for monitoring the progress of patients
   e. Immunoassay measurement is made of **β-hCG.**
6. **CA 15-3**
   a. Mucin glycoprotein antigen
   b. Useful for monitoring therapeutic response and for detecting recurrence of **breast cancer** in patients previously treated
   c. Elevated levels are observed in nonmalignant diseases such as chronic hepatitis, tuberculosis, and systemic lupus erythematosus.
   d. Immunoassay methods are used for measurement.
7. **CA 125**
   a. Mucin glycoprotein antigen
   b. Marker for **ovarian** and endometrial cancer
   c. Useful for monitoring the progress of patients
   d. Immunoassay methods are used for measurement.
8. **CA 19-9**
   a. Glycolipid blood group antigen-related marker; sialylated derivative of the Lewis blood group system, known as **Le^{xa}**
   b. Marker for **pancreatic, colorectal,** lung, and gastric carcinomas
   c. Useful for monitoring the progress of patients
   d. Immunoassay methods are used for measurement.

III. NONPROTEIN NITROGENOUS COMPOUNDS

A. Urea

1. **Regulation:** Urea is the **major nitrogen-containing compound** in the blood. It results from protein catabolism and is synthesized in the liver from the deamination of amino acids. Urea is **excreted by the kidneys.**

2. **Clinical significance:** Abnormal serum urea levels may be due to prerenal, renal, or postrenal disorders.
   a. **Increased serum urea:** Renal failure, glomerular nephritis, urinary tract obstruction, congestive heart failure, dehydration, increased protein catabolism
   b. **Decreased serum urea:** Severe liver disease, vomiting, diarrhea, malnutrition
3. Blood urea nitrogen (BUN) is an older term still in use, and the terminology was based on previous methodology where nitrogen was measured. To convert BUN to urea: BUN $\times$ 2.14 = Urea.
4. **Test methodology**
   a. **Kinetic method**

$$\text{Urea} + 2H_2O + H^+ \xrightarrow{\text{urease}} (NH_4)_2CO_3 + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{glutamate dehydrogenase}} \text{glutamate} + NAD^+ + H^+$$

   b. **Chemical method**

$$\text{Diacetyl monoxime} + H_2O \rightarrow \text{diacetyl} + \text{hydroxylamine}$$

$$\text{Diacetyl} + \text{urea (acid medium)} \rightarrow \text{diazine derivative (yellow)}$$

5. **Reference range:** 6–20 mg/dL

B. Creatinine

1. **Regulation:** Creatinine is a waste product of muscle contraction that is formed from phosphocreatine, a high-energy compound. Creatinine levels are **regulated by kidney excretion.** Measurements of creatinine in serum and urine (creatinine clearance) are used to assess the glomerular filtration rate (GFR). Creatinine levels are not changed by diet or rate of urine flow. Creatinine is not reabsorbed by renal tubules.
2. **Clinical significance**
   a. **Increased serum creatinine:** Renal disease, renal failure
3. **Test methodology**
   a. **Jaffe method**

$$\text{Creatinine} + \text{picric acid (alkaline solution)} \rightarrow$$

$$\text{creatinine picrate (red tautomer)}$$

   b. **Enzymatic method**

$$\text{Creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{sarcosine oxidase}} \text{glycine} + \text{formaldehyde} + H_2O_2$$

$$H_2O_2 + \text{indicator (reduced)} \xrightarrow{\text{peroxidase}} \text{indicator (oxidized)} + 2H_2O$$

4. **Reference ranges:** Male, 0.9–1.3 mg/dL; female, 0.6–1.1 mg/dL
5. **Creatinine clearance** is used to **assess** the **GFR.** Testing requires a plasma sample and a 24-hour urine collection.
   a. **P:** plasma creatinine mg/dL, **U:** urine creatinine mg/dL, **V:** urine flow in mL/min, and **SA:** body surface area; 1.73 m^2 = average body surface area
   b. **Creatinine clearance formula:**

$$C\ (mL/min) = \frac{U \times V}{P} \times \frac{1.73\ m^2}{SA}$$

   c. **Reference ranges:** Differ according to age and sex; values decrease with age
   Creatinine clearance (males): 105 ± 20 mL/min/1.73 m^2
   Creatinine clearance (females): 95 ± 20 mL/min/1.73 m^2
   d. **Estimated glomerular filtration rate (eGFR)** uses only a **blood creatinine** and the **MDRD** (Modification of Diet in Renal Disease) **formula.**
      1) Correction for gender and race required
      2) Results only reported as a number if <60 mL/min/1.73 m^2

C. Uric Acid

1. **Regulation:** Uric acid, the major waste product of purine (adenosine and guanine) catabolism, is synthesized in the liver. Uric acid **elimination** from the blood is **regulated by the kidneys** through glomerular filtration, and some uric acid is excreted through the GI tract.
2. **Clinical significance**
   a. **Increased serum uric acid:** Gout, renal disorders, treatment of myeloproliferative disorders, lead poisoning, lactic acidosis, toxemia of pregnancy, Lesch-Nyhan syndrome
   b. **Decreased serum uric acid:** Severe liver disease as a secondary disorder, tubular reabsorption disorders, drug induced
3. **Test methodology**
   a. **Chemical method**

$$\text{Uric acid} + \text{phosphotungstic acid} + O_2 \rightarrow \text{allantoin} + CO_2 + \text{tungsten blue}$$

   b. **Enzymatic uricase method:** Decrease in absorbance monitored at 293 nm

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{allantoin} + H_2O_2 + CO_2$$

4. **Reference ranges:** Male, 3.5–7.2 mg/dL; female, 2.6–6.0 mg/dL

D. Ammonia

1. **Regulation**
   a. Ammonia produced from **deamination of amino acids**
   b. Hepatocytes convert ammonia **to urea** for excretion.
   c. With severe liver cell malfunction, blood levels of ammonia **increase.**
   d. Ammonia is neurotoxic.
2. **Type of specimen and storage**
   a. Venous blood free of hemolysis; **place on ice immediately**
   b. Blood collected in ethylenediaminetetra-acetic acid (EDTA) may be used.
   c. Centrifuge sample within 20 min of collection and remove plasma.
   d. Plasma stable up to 3½ hr in ice bath; stable several days frozen
3. **Clinical significance:** Increased plasma ammonia levels seen in hepatic failure and Reye syndrome
4. **Test methodology**

$$\alpha\text{-Ketoglutarate} + NH_4^+ + NADPH \xrightarrow[\text{dehydrogenase}]{\text{glutamate}} \text{Glutamate} + NADP^+ + H_2O$$

5. **Interferences**
   a. Incorrect handling of blood sample
   b. Ammonia contamination
6. **Reference range:** 11–32 μmol/L

IV. CARBOHYDRATES

A. Glucose Metabolism

1. During a fast, the blood glucose level is kept constant by mobilizing the **glycogen** stores in the liver.
2. During long fasts, **gluconeogenesis** is required to maintain blood glucose levels because glycogen stores are used up in about 24–48 hours.
3. An individual with a **fasting blood glucose level >100 mg/dL** is referred to as **hyperglycemic.** An individual with a **fasting blood glucose level <50 mg/dL** is referred to as **hypoglycemic.**

B. Hormones Affecting Blood Glucose Levels

1. **Insulin:** Produced by the beta cells of the pancreatic islets of Langerhans; promotes the entry of glucose into liver, muscle, and adipose tissue to be stored as glycogen and fat; inhibits the release of glucose from the liver
2. **Somatostatin:** Synthesized by delta cells of the pancreatic islets of Langerhans; inhibits secretion of insulin, glucagon, and growth hormone, resulting in an increase in plasma glucose level
3. **Growth hormone** and adrenocorticotropic hormone (**ACTH**): Hormones secreted by the anterior pituitary that raise blood glucose levels

4. **Cortisol:** Secreted by the adrenal glands; stimulates glycogenolysis, lipolysis, and gluconeogenesis
5. **Epinephrine** is secreted by the medulla of the adrenal glands. It stimulates glycogenolysis and lipolysis; it inhibits secretion of insulin. Physical or emotional stress causes increased secretion of epinephrine and an immediate increase in blood glucose levels.
6. **Glucagon:** Secreted by the α cells of the pancreatic islets of Langerhans; increases blood glucose by stimulating glycogenolysis and gluconeogenesis
7. **Thyroxine:** Secreted by the thyroid gland; stimulates glycogenolysis and gluconeogenesis; increases glucose absorption from the intestines

C. Renal Threshold for Glucose

1. Glucose is filtered by the glomeruli, reabsorbed by the tubules, and normally **not** present in urine. If the blood glucose level is elevated, glucose appears in the urine, a condition known as **glucosuria.**
2. An individual's **renal threshold** for glucose varies between **160 and 180 mg/dL.** When blood glucose reaches this level or exceeds it, the renal tubular transport mechanism becomes saturated, which causes glucose to be excreted into the urine.

D. Abnormal Carbohydrate Metabolism

1. **Classification of diabetes mellitus**
   a. **Type 1 diabetes mellitus**
      1) Characterized by **insulinopenia,** a deficiency of insulin
      2) Individuals require **treatment with insulin** to sustain life.
      3) Most individuals exhibit it as an autoimmune disorder where β cells of the islets of Langerhans are destroyed by the body.
      4) Peak incidence is in childhood and adolescence, but it may occur at any age.
      5) Primary symptoms include polyuria, polydipsia, and weight loss.
      6) **Ketosis-prone:** Can produce excess ketones, resulting in diabetic ketoacidosis
   b. **Type 2 diabetes mellitus**
      1) Defect in insulin secretion and cellular resistance to insulin
      2) Individuals are **not dependent on treatment with insulin.** Individuals generally respond to dietary intervention and oral hypoglycemic agents, but some may require insulin therapy.
      3) Associated with obesity and sedentary lifestyle; symptoms include polyuria, polydipsia, and weight loss
      4) Although associated with individuals over the age of 40, type 2 diabetes mellitus is becoming a significant problem in children and adolescents.
      5) **Non–ketosis prone:** Without exogenous insulin or oral hypoglycemic medication, these individuals will have an elevated glucose but will not go into diabetic ketoacidosis.

c. **Gestational diabetes mellitus (GDM)**
   1) GDM is the onset of diabetes mellitus during **pregnancy.**
   2) After childbirth, the individual generally returns to normal metabolism. However, there is an increased chance that type 2 diabetes mellitus may develop later in life.

2. **Inherited disorders of carbohydrate metabolism**
   a. **Glycogen storage diseases,** of which there are 10 types, are inherited diseases involving the deficiency of particular enzymes; these deficiencies cause defects in the normal metabolism of glycogen.
      1) **von Gierke, type I:** Glucose-6-phosphatase deficiency
      2) **Pompe, type II:** α-1,4-glucosidase deficiency
      3) **Cori, type III:** Amylo-1,6-glucosidase deficiency
   b. **Galactosemia**
      1) This is characterized by a deficiency or absence of galactokinase, galactose 1-phosphate uridyl transferase, or uridyl diphosphate glucose-4-epimerase; the enzyme defect prevents metabolism of galactose. Galactose is found in milk as a component of lactose, with galactosemia generally identified in infants.
      2) Most commonly, **galactose 1-phosphate uridyl transferase** is deficient, which leads to excessive galactose in blood and excretion in urine.

E. Laboratory Diagnosis

1. **Normal** fasting plasma glucose (FPG) **<100 mg/dL**
2. **Impaired fasting glucose (IFG)** is defined as a fasting plasma glucose level that **ranges between 100 and 125 mg/dL.**
3. **Provisional diagnosis of diabetes mellitus** is made when **FPG ≥126 mg/dL.** The diagnosis **must be confirmed** by one of the three methods described in the following outline section.
4. **Diagnosis of diabetes mellitus**
   a. A plasma glucose analysis that yields **any one** of the following results is **diagnostic for the presence of diabetes mellitus,** provided that unequivocal hyperglycemia is apparent. If unequivocal hyperglycemia is not apparent, the **glucose result must be confirmed** by repeat analysis on a subsequent day using any one of the following three methods. However, the American Diabetes Association does **not** recommend the oral glucose tolerance test (OGTT) for routine clinical use.
      1) An individual expressing physical symptoms and a **casual plasma glucose level of ≥200 mg/dL**
      2) **Fasting plasma glucose** level that is **≥126 mg/dL** (fasting defined as no caloric intake for minimum of 8 hours)
      3) Plasma glucose level of **≥200 mg/dL at 2-hour point** of an **OGTT** as described by the World Health Organization (WHO)
   b. **Gestational diabetes mellitus (GDM)**

1) A woman at **high risk** for GDM should have an **initial screening early** in the pregnancy. If she is not found to have GDM during the initial screening, the woman should be **retested at 24 to 28 weeks** of gestation. For women of **average risk,** testing should be performed at 24 to 28 weeks of gestation.
2) For **GDM, fasting** plasma glucose **≥126 mg/dL** or a **casual** plasma glucose **≥200 mg/dL** is diagnostic of diabetes mellitus.
3) If unequivocal hyperglycemia is not apparent, **retesting** must be performed on a subsequent day.
4) When using the **two-step approach,** an **initial screening** is performed using a **50-g oral glucose load** (time of day or time of last meal not relevant). Plasma is **tested at 1 hour.** This is a glucose challenge test (GCT). If the test value exceeds the glucose threshold value **≥140 mg/dL,** an **OGTT is performed.** Some experts recommend using a glucose threshold value of ≥130 mg/dL.
5) **Gestational diabetes mellitus** may be diagnosed using an **OGTT** with oral ingestion of **100 g of glucose.** The glucose results must meet or exceed **two or more** of the following **criteria:** a fasting plasma glucose >95 mg/dL, a 1-hour plasma glucose >180 mg/dL, a 2-hour plasma glucose >155 mg/dL, or a 3-hour plasma glucose >140 mg/dL. Alternatively, a 75-g glucose load may be used and glucose measured through the 2-hour period.

5. **Oral glucose tolerance test** based on the criteria published by the World Health Organization (WHO). *Note:* American Diabetes Association does **not** recommend the OGTT for routine clinical use.
   a. Timed measurements of plasma glucose before and after ingesting a specific amount of glucose
   b. **Patient preparation:** Unrestricted carbohydrate rich diet for 3 days before the test with physical activity, restrict medication on the test day, 12-hour fast required, no smoking
   c. Adult patient ingests 75 grams of glucose in 300–400 mL of water and children 1.75 g/kg up to 75 g of glucose. For assessment of GDM, 50 g, 75 g, or 100 g of glucose may be used (see previous description for details).
   d. Plasma glucose specimen is collected fasting at 10 minutes before glucose load and at 120 minutes after ingestion of glucose. Urine glucose may be measured.
   e. **Interpretation of OGTT results** is based on the criteria published by the WHO.
      1) **Impaired fasting glucose (IFG)** is diagnosed when fasting plasma glucose ranges between 110 and 125 mg/dL.
      2) The following two criteria must be met for diagnosis of **impaired glucose tolerance (IGT):** Fasting plasma glucose level must be

≤126 mg/dL and the 2-hour plasma glucose level of the OGTT must fall between 140 and 199 mg/dL.

3) **Diabetes mellitus** is diagnosed when the fasting plasma glucose level is ≥126 mg/dL or the 2-hour glucose is ≥200 mg/dL.

6. **Glycated/glycosylated hemoglobin**
   a. Hemoglobin A is composed of three forms, Hb A_{1a}, Hb A_{1b}, and Hb A_{1c}, which are referred to as **glycated** or **glycosylated hemoglobin. Hb A_{1c}** is the **main form.**
   b. Glycated hemoglobin is formed from the **nonenzymatic, irreversible attachment of glucose** to hemoglobin A_1.
   c. Measurement of glycated hemoglobin **reflects blood glucose levels for the past 2–3 months.** It is useful in monitoring effectiveness of treatment and compliance of diabetic individual to treatment protocol.
   d. **Measured** by affinity chromatography, ion-exchange chromatography, and high-performance liquid chromatography
   e. **Specimen collection:** Nonfasting blood drawn in EDTA tubes
   f. **Reference range:** 4–6% Hb A_{1c}; effective treatment range <7% Hb A_{1c}
7. **Fructosamine**
   a. Ketoamine linkage forms between glucose and protein, mainly represented by albumin.
   b. **Clinical significance:** Measurement of fructosamine **reflects blood glucose levels for 2–3 weeks** before sampling.
   c. **Measured** by spectrophotometric/colorimetric methods, affinity chromatography, and high-performance liquid chromatography
   d. **Reference range:** 205–285 μmol/L
8. Measurement of albumin excretion is useful for patients with **renal complications of diabetes mellitus.** Performed on random urines, microalbumin analysis always requires the simultaneous analysis of creatinine, and it is reported as an albumin/creatinine ratio. Abnormal values **(microalbuminuria)** will be **≥30 mg albumin/g creatinine.**

F. Measurement of Plasma Glucose

1. **Glucose oxidase method**

$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen (colored)} + H_2O$$

2. **Hexokinase method**

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + NADP^+ \xrightarrow[\text{dehydrogenase}]{\text{glucose-6-phosphate}} \text{6-phosphogluconate} + NADPH + H^+$$

3. **Clinical significance:** An increase in the blood glucose level is the hallmark of diabetes mellitus, but it can also be indicative of other hormonal disorders such as Cushing disease.
4. **Reference range:** Adult fasting, 74–99 mg/dL

G. Lactate

1. The normal end product of glucose metabolism is pyruvate; however, lactate is produced under conditions of oxygen deficit (anaerobic metabolism). The production and accumulation of lactate in the blood and its measurement aid in assessing the degree of oxygen deprivation that is occurring. Change in the blood lactate level precedes a change in blood pH. Lactate is metabolized by the liver via gluconeogenesis.
2. **Test methodology**

$$\text{Lactate} + O_2 \xrightarrow{\text{lactate oxidase}} \text{pyruvate} + H_2O_2$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen (colored)} + H_2O$$

3. **Clinical significance: Type A lactic acidosis** is caused by depressed oxygen levels that may occur in acute myocardial infarction, congestive heart failure, shock, pulmonary edema, and so on. **Type B lactic acidosis** is caused by metabolic processes that may occur in diabetes mellitus, renal disorders, liver disease, ingestion of toxins (salicylate overdose and excess ethanol), and so on.
4. **Special specimen handling** is required and includes the following: Avoid using a tourniquet because venous stasis will falsely raise blood lactate levels; place the specimen on ice and immediately transport to the laboratory; centrifuge the specimen and remove the plasma (additives NaF and $K_2C_2O_4$) as soon as possible.
5. **Reference range (venous):** 0.5–1.3 mmol/L

V. LIPIDS AND LIPOPROTEINS

A. Lipid Structure

1. **Fatty acids** exist as short, medium, and long chains of molecules that are major constituents of triglycerides and phospholipids. Minimal amounts of fatty acids are bound to albumin and circulate free (unesterified) in plasma.
2. **Triglyceride**
   a. Triglyceride is formed from one glycerol molecule with three fatty acid molecules attached via ester bonds.
   b. Triglycerides comprise 95% of all fats stored in adipose tissue.
   c. Triglycerides are **transported** through the body by **chylomicrons** and **VLDL** (very-low-density lipoprotein).
   d. Metabolism involves releasing the fatty acids to the cells for energy, then recycling the glycerol into triglyceride.

e. Lipase, lipoprotein lipase, epinephrine, and cortisol break down triglycerides.

3. **Cholesterol**
   a. Unsaturated steroid alcohol; exists in the **esterified** form, where a fatty acid forms an ester bond at carbon-3, and the **free** (unesterified) form
   b. **Precursor for synthesis** of bile acids, steroid hormones, and vitamin D
   c. Low-density lipoprotein (LDL) is the primary carrier of cholesterol.
4. **Phospholipid**
   a. Composed of one glycerol molecule and two fatty acid molecules attached via ester bonds
   b. Found on the surface of lipid layers, they are major constituents of cell membranes and outer shells of lipoprotein molecules.

B. Classification of Lipoproteins

1. **Lipoproteins** are molecules that combine water insoluble dietary lipids and water-soluble proteins (apolipoproteins) so that lipids can be transported throughout the body. Micelles are spherical and have an inner core of neutral fat.
2. **Chylomicrons** are the largest lipoproteins and have the lowest density. They are formed in the intestines and transport **triglycerides** after a meal, giving serum a turbid appearance. Because of their low density, chylomicrons will float to the top and form a creamy layer when plasma is stored overnight. **Chylomicrons** are composed of **86% triglyceride,** 5% cholesterol, 7% phospholipid, and 2% apolipoprotein. Chylomicrons have apoproteins **B-48,** mainly, and lesser amounts of A-I, C-I, C-II, and C-III on their surface. In **normal lipid metabolism,** chylomicrons enter the circulation and are metabolized to remnant particles for uptake and further modification by the liver.
3. **Very-low-density lipoprotein** carries endogenous **triglycerides** synthesized in the liver. **VLDL** molecules are composed of **55% triglycerides,** 19% cholesterol, 18% phospholipid, 8% apolipoprotein and have apolipoproteins **B-100,** mainly, and C-I, C-II, C-III, and E on their surface. In **normal lipid metabolism,** VLDLs are secreted into the blood by the liver for metabolism in peripheral tissues.
4. **Intermediate-density lipoprotein** (IDL) is a transitional form, as it is **formed from VLDL** and then further **modified** in the liver **to LDL.** IDLs carry endogenous triglycerides and cholesterol esters. IDL molecules are composed of 23% triglycerides, 38% cholesterol, 19% phospholipid, 19% apolipoprotein and have apolipoproteins **B-100,** mainly, and some E on their surface.
5. **Low-density lipoprotein** is the body's **major cholesterol carrier** and transports a large amount of endogenous cholesterol. Known as "**bad cholesterol,**" LDL is easily taken up by cells, so elevated levels are associated with increased risk for atherosclerosis. **LDLs** are composed of **50% cholesterol,** 22% phospholipids, 6% triglycerides, and 22% protein and have apoprotein **B-100** on their surface. In **normal lipid metabolism,** this lipoprotein

brings cholesterol to peripheral cells for membrane synthesis and formation of adrenal and reproductive hormones.

6. **High-density lipoprotein** (HDL) is also known as **"good cholesterol." HDL** is synthesized in the intestine and liver cells. **HDL** molecules are recycled chylomicron and VLDL molecules. **HDL** is composed of **50% protein,** 28% phospholipids, 19% cholesterol, and 3% triglycerides. HDL has apoproteins **A-I,** mainly, and A-II on its surface. In **normal lipid metabolism,** HDL removes excess cholesterol from peripheral tissues and transports it to other catabolic sites. This function has an **antiatherogenic effect.**
7. **Lp(a)** is composed primarily of cholesterol esters, phospholipids, and **apolipoprotein (a)** and B-100. **Elevated** levels of Lp(a) are associated with **increased risk for coronary heart disease,** myocardial infarction, and cerebrovascular disease.

C. Clinical Significance

1. Abnormal lipid metabolism can be due to genetic defects or it can be acquired. Abnormal lipid metabolism is associated with risk of coronary heart disease and other disorders.
2. The National Cholesterol Education Program established the Adult Treatment Panel III Classification (ATP III), which sets cutoff values for cholesterol and triglyceride levels based on a 9- to 12-hour fast. See Tables 1-1■ through 1-4■.

TABLE 1-1 TOTAL CHOLESTEROL REFERENCE RANGE

	Desirable	Borderline High	High
Total Cholesterol (mg/dL)	<200	200–239	≥240

TABLE 1-2 HDL CHOLESTEROL REFERENCE RANGE

	Protective against Heart Disease	The Higher, the Better	Major Risk Factor for Heart Disease
HDL Cholesterol (mg/dL)	≥60	40–59	<40

TABLE 1-3 LDL CHOLESTEROL REFERENCE RANGE

	Optimal	Near Optimal	Borderline High	High	Very High
LDL Cholesterol (mg/dL)	<100	100–129	130–159	160–189	≥190

TABLE 1-4 TRIGLYCERIDE REFERENCE RANGE

	Normal	Borderline High	High	Very High
Triglyceride (mg/dL)	<150	150–199	200–499	≥500

3. **Hyperlipoproteinemias** have been classified using the Fredrickson-Levy classification system, which is not commonly used today. However, some of the abnormal lipid types are still referenced in the literature and for that reason are included here.
   a. **Type I hyperlipoproteinemia: Elevated chylomicrons**
      1) Serum appearance: Creamy layer of chylomicrons over clear serum
      2) Total cholesterol: Normal to moderately elevated
      3) Triglyceride: Extremely elevated
      4) Apo B-48 increased, Apo A-IV increased
   b. **Type IIa hyperlipoproteinemia: Increased LDL**
      1) Serum appearance: Clear
      2) Total cholesterol: Generally elevated
      3) Triglyceride: Normal
      4) Apo-B 100 increased
   c. **Type IIb hyperlipoproteinemia: Increased LDL and VLDL**
      1) Serum appearance: Clear or slightly turbid
      2) Total cholesterol: Elevated
      3) Triglyceride: Elevated
      4) Apo B-100 increased
   d. **Type III hyperlipoproteinemia: Increased IDL**
      1) Serum appearance: Creamy layer sometimes present over a turbid layer
      2) Total cholesterol: Elevated
      3) Triglyceride: Elevated
      4) Apo E-II increased, Apo E-III decreased, and Apo E-IV decreased
   e. **Type IV hyperlipoproteinemia: Increased VLDL**
      1) Serum appearance: Turbid
      2) Total cholesterol: Normal to slightly elevated
      3) Triglyceride: Moderately to severely elevated
      4) Apo C-II either increased or decreased, and Apo B-100 increased
   f. **Type V hyperlipoproteinemia: Increased VLDL with increased chylomicrons**
      1) Serum appearance: Turbid with creamy layer
      2) Total cholesterol: Slightly to moderately elevated
      3) Triglyceride: Severely elevated
      4) Apo C-II increased or decreased, Apo B-48 increased, and Apo B-100 increased

4. The most common familial form is **familial combined hyperlipidemia (FCHL).** FCHL is characterized by increased plasma levels of total and LDL cholesterol (type IIa), or triglyceride (type IV), or a combination of both (type IIb). Also, apo B-100 is increased. The level of HDL cholesterol may be decreased.
5. **Hyperapobetalipoproteinemia** is associated with VLDL and apo B-100 overproduction in the liver. It is characterized by normal or moderate elevation of LDL cholesterol with an elevated apo B-100. Total cholesterol and triglyceride are generally elevated but may be normal. HDL cholesterol and apo A-I levels are decreased.
6. **Familial hypertriglyceridemia** is characterized by a moderate elevation of triglyceride with excess production of VLDL. Both triglyceride and cholesterol are present in higher concentrations than normal in VLDL. LDL cholesterol and apo B-100 are within their reference ranges. HDL cholesterol is decreased.
7. **Type V hyperlipoproteinemia** is characterized by increased VLDL and chylomicrons.
8. **Familial hypercholesterolemia** is characterized by increased LDL cholesterol. The plasma triglyceride level may be normal or slightly increased, and the plasma HDL cholesterol level is slightly decreased.
9. **Secondary lipoproteinemia:** Many conditions cause lipoproteins to be abnormally metabolized. Some of those conditions include diabetes mellitus, hypothyroidism, obesity, pregnancy, nephrotic syndrome, pancreatitis, alcoholism, and myxedema.
10. **Hypolipoproteinemias**
    a. **Abetalipoproteinemia:** Total cholesterol level very low, triglyceride level nearly undetectable, LDL and Apo B-100 absent
    b. **Hypobetalipoproteinemia:** Unable to synthesize apo B-100 and apo B-48, low total cholesterol level and normal to low triglyceride level
    c. **Hypoalphalipoproteinemia:** Severely elevated triglyceride level and low HDL level
    d. **Tangier disease:** HDL absent, apo A-I and apo A-II very low levels, LDL low, total cholesterol level low, triglyceride level normal to slightly increased

D. Cholesterol Test Methodology

1. **Elevated** cholesterol concentrations have been linked to atherosclerosis, coronary artery disease, and increased risk for myocardial infarction.
2. **Decreased** cholesterol levels are present in various forms of liver disease, most notably alcoholic cirrhosis.
3. **Enzymatic methodology**

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{cholesteryl esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 2H_2O$$

E. HDL Cholesterol Test Methodology

1. **HDL decreases the atherosclerotic process.** Increased HDL cholesterol is associated with **decreased risk** of coronary artery disease, and decreased HDL cholesterol is associated with **increased risk** of coronary artery disease.
2. **Test methodology**
   a. **Precipitate LDL and VLDL** cholesterol with dextran sulfate-magnesium chloride or heparin sulfate-manganese chloride, then **assay the supernatant** for cholesterol using an enzymatic technique. **Cholesterol present is HDL.**
   b. **Homogeneous assay** uses an antibody to apo B-100 to bind LDL and VLDL. An enzymatic cholesterol analysis can then be performed with only HDL cholesterol able to react.

F. LDL Cholesterol Test Methodology

1. **LDL** is directly **associated with artherosclerosis and coronary heart disease.**
2. **Test methodology:** LDL cholesterol may be **calculated or measured directly.**
   a. **Friedewald formula** (indirect, not valid for triglycerides over 400 mg/dL):

LDL cholesterol = total cholesterol − [HDL cholesterol + triglyceride/5]

   b. **Homogeneous** assay uses detergents to block HDL and VLDL from reacting with the dye to form a colored chromogen product. An enzymatic cholesterol analysis is performed with only LDL cholesterol able to react.

G. Triglyceride Test Methodology

1. **Elevated** triglyceride levels may be seen in Fredrickson Type I, IIb, IV, and V hyperlipoproteinemias, pancreatitis, alcoholism, obesity, hypothyroidism, nephrotic syndrome, and storage diseases (Gaucher, Niemann-Pick).
2. **Enzymatic methodology**

$$\text{Triglyceride} + 3\,H_2O \xrightarrow{\text{lipase}} \text{glycerol} + 3 \text{ fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{glycerophosphate} + \text{ADP}$$

$$\text{Glycerophosphate} + O_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{dihydroxyacetone} + H_2O_2$$

$$H_2O_2 + \text{Dye} \xrightarrow{\text{peroxidase}} \text{colored product} + 2H_2O$$

H. Apo A-1, Apo B, and Lp(a)

1. **Clinical significance**
   a. **Apo A-1** is the major protein found in HDL. It activates lecithin-cholesterol acyltransferase (LCAT) and removes free cholesterol from extrahepatic tissues. Thus, it is considered **antiatherogenic.**
   b. **Apo B-100** is the major protein found in LDL. It is **associated with increased risk of coronary artery disease.**
   c. **Lp(a)** is an independent risk factor associated with impaired plasminogen activation and thus decreased fibrinolysis. A high level suggests **increased risk for coronary heart disease and stroke.**
2. **Test methodology**
   a. Apo-A, Apo-B, and Lp(a) are measured by immunochemical methods such as immunoturbidimetric and immunonephelometric.
3. **Reference ranges**
   a. **Apo-A:** 120–160 mg/dL
   b. **Apo-B:** <120 mg/dL
   c. **Lp(a):** <30 mg/dL

VI. ENZYMES AND CARDIAC ASSESSMENT

A. General Properties

1. **Definition: Enzymes** are **proteins** that function as **biological catalysts** and are neither consumed nor permanently altered during a chemical reaction. They appear in the serum in increased amounts after cellular injury or tissue damage.
2. **Isoenzyme:** These are **different forms** of the same enzyme capable of the **same catalytic function** in the body. Isoenzymes may be differentiated based on electrophoretic mobility and resistance to heat denaturation.
3. **Cofactor:** A nonprotein compound that may be required for enzyme activity
4. **Activators:** Inorganic cofactors needed for enzymatic activity, such as Mg^{2+}, Zn^{2+}, or Cl^-
5. **Coenzyme:** Organic cofactor, such as NAD^+ (nicotinamide adenine dinucleotide)
6. **Prosthetic group:** Organic cofactor tightly bound to the enzyme
7. **Active site:** Location on an enzyme where the three-dimensional arrangement of amino acid residues allows binding of substrate
8. **Denaturation:** Causes change in enzyme structure that results in loss of activity; may be caused by elevated temperature, extreme change in pH, and certain chemicals

B. Enzyme Kinetics

1. **Activation energy** is the energy required to raise all molecules to the transition state in a chemical reaction so that products may be formed.
2. **Enzymes** increase the rate of chemical reactions by lowering the activation energy required by substrate to react and form the product.

C. Factors That Influence Enzyme Reactions

1. **Substrate concentration**
   a. **Substrate** binds to free enzyme **at low substrate concentration.** As long as the enzyme exceeds the amount of substrate, the reaction rate increases as more substrate is added. The rate of the reaction is directly proportional to substrate concentration **(first-order kinetics).**
   b. When the **substrate concentration is high** enough to bind with all available enzyme, the **reaction velocity is at its maximum.** As product is formed, the enzyme becomes available to react with additional substrate **(zero-order kinetics).** When excess substrate is present, the rate of the reaction depends only on the concentration of enzyme.
2. **Enzyme concentration:** The reaction velocity is proportional to the enzyme concentration, provided that substrate concentration exceeds enzyme concentration.
3. **pH:** It is important that pH be controlled, because extreme pHs can denature an enzyme or change its ionic state and, possibly, the reactivity of the active site. Most enzymes of physiological interest function at pH 7.0–8.0.
4. **Temperature:** An increase in temperature will increase the rate of a chemical reaction. In general, the rate of an enzymatic reaction will double with each 10°C increase in temperature, until the rise in temperature causes the enzyme to denature. Enzymes have an optimal reaction temperature, which is usually 37°C. Denaturation generally occurs at 40–50°C.
5. **Inhibitors**
   a. A substance that **interferes** with an enzyme-catalyzed reaction
   b. **Competitive inhibitor** competes with substrate for the active site. This inhibition is reversible.
   c. **Noncompetitive inhibitor** binds with the enzyme at a site different from the active site and prevents the enzyme-catalyzed reaction from taking place. This inhibition may be reversible or irreversible. It may be irreversible if the active site is affected.
   d. **Uncompetitive inhibitor** binds to the enzyme-substrate complex so that increasing the concentration of substrate leads to the formation of more enzyme-substrate complexes and more inhibition.

D. Measuring Enzyme Activity

1. Enzyme reactions are performed in **zero-order kinetics,** with substrate in excess.

2. It is extremely important in enzyme reactions that the pH and temperature **remain constant,** and that additives (e.g., cofactors, coenzymes, activators) are present in sufficient amounts.
3. There are **two methods** used to measure enzyme reactions: endpoint and kinetic.
   a. **Endpoint:** This type of reaction combines reactants, stops the reaction at a fixed time (e.g., 20 minutes), and then measures the product formed. Activity of the enzyme is based on the final absorbance reading.
   b. **Kinetic:** This type of reaction combines reactants, then measures the change in absorbance at specific time intervals (e.g., 60 sec) over a specific time period. Activity of the enzyme is based on the change in absorbance over time.

E. Calculation of Enzyme Activity

1. Enzymes are reported in **activity units** because they are measured based on their activity instead of their concentration.
2. **International unit (IU or U)** is the quantity of enzyme that catalyzes the reaction of one micromole of substrate per minute under specified conditions, including temperature, pH, substrates, and activators. Results are generally reported as IU/L, U/L, or mU/mL.

F. Specific Enzymes of Clinical Interest

1. In general, each enzyme of clinical significance is found in **many tissues** of the body, and in healthy individuals, these enzymes exhibit **very low levels in serum.** In certain disease states or with cell injury, these **intracellular enzymes** are **released into the blood** and are indicative of the **presence of a pathological condition.** Quantification of enzyme levels in serum is useful in determining the presence of disease. Based on the individual's physical symptoms, several enzymes may be chosen for analysis to determine if a pattern develops that aids in identifying the tissue source of the enzyme elevation in the serum.
2. **Lactate dehydrogenase (LD)**
   a. **Tissue location**
      1) **Highest concentrations:** Liver, heart, skeletal muscle, kidney, erythrocytes, with lesser amounts in many other tissues
      2) **LD isoenzymes**
         a) LD isoenzymes consist of **four subunits** (polypeptide chains) derived from two types of polypeptides designated **M** (muscle/liver) and **H** (heart).
         b) Each LD isoenzyme is a tetramer with five isoenzyme types: LD-1 through LD-5. LD-1 and LD-2 are associated with acute myocardial infarction (AMI) and erythrocyte destruction. LD-3 is associated with pulmonary disorders, pancreatitis, and lymphocytosis. LD-4 and LD-5 are associated with liver and skeletal muscle disorders.

b. **Clinical significance**
   1) **Elevated** in cardiac disorders (acute myocardial infarction), hepatic diseases (viral hepatitis, cirrhosis, infectious mononucleosis), skeletal muscle diseases, hemolytic and hematologic disorders (pernicious anemia exhibits extreme elevation of LD), and neoplastic disorders (acute lymphoblastic leukemia)
   2) In **AMI,** LD levels rise within 8–12 hours, peak at 24–48 hours, and return to normal in 7–10 days. Although LD and LD isoenzymes are **not used to diagnose AMI,** knowledge of their pattern may be **useful when assessing concurrent liver damage.**

c. **Test methodology**

$$\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LD (pH 8.3-8.9)}} \text{pyruvate} + \text{NADH} + \text{H}^+$$

   1) **Sources of error:** Hemolysis; LD-4 and LD-5 labile at 4°C
   2) **Reference range:** 100–225 U/L at 37°C

3. **Creatine kinase (CK) and CK isoenzymes**
   a. **Tissue location**
      1) **Highest concentrations:** Skeletal muscle, heart muscle, brain tissue
      2) **CK isoenzymes**
         a) CK isoenzymes consist of **two subunits: M** for muscle and **B** for brain.
         b) Each CK isoenzyme is a dimer with three possible types: **CK-MM** (or CK-3), **CK-MB** (or CK-2), and **CK-BB** (or CK-1).
         c) In serum, healthy individuals have **CK-MM** as the **major isoenzyme** and a small amount of **CK-MB** (less than 6% of total CK), whereas **CK-BB** is **not normally detectable.**
         d) **CK-MB** increases are associated with heart muscle damage, and elevations are indicative of AMI when used in conjunction with other markers, such as troponin. However, CK-MB also increases in other disorders, such as skeletal muscle damage. **CK-MM** increases are associated with skeletal muscle and heart muscle disorders. **CK-BB** is elevated in central nervous system disorders and tumors of various organs, including the prostate gland.

   b. **Clinical significance**
      1) Elevations of **total CK** in serum are associated with cardiac disorders, such as AMI, and skeletal muscle disorders, such as muscular dystrophy. Occasionally, elevations are due to disorders of the central nervous system, including seizures and cerebral vascular accidents.
      2) **CK-MB** values **greater than 6%** of total CK are **suggestive of AMI.** When AMI is suspected, **troponin** is assayed in conjunction with CK-MB, and sometimes **myoglobin** is assayed. **Following AMI, CK-MB**

levels rise within 4–6 hours, peak at 12–24 hours, and return to normal within 2–3 days.

c. **Test methodology**

1) **CK isoenzymes** are measured by electrophoresis, ion-exchange chromatography, and several types of immunoassays. Immunoassays that measure **enzyme mass** are more sensitive than activity-based assays.

2) **Methodology**

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow[Mg^{2+}]{\text{creatine kinase}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G6PD}} \text{6-phosphogluconate} + NADPH + H^+$$

a) **Sources of error:** Moderate hemolysis

b) **Reference ranges:**
Total CK: male, 15–160 U/L; female, 15–130 U/L at 37°C
CK-MB: <6% of total CK; mass assay 0–5 ng/mL

4. **Aspartate aminotransferase (AST)**

a. **Tissue location:** Highest concentrations in heart, liver, and skeletal muscle, with lesser amounts in kidney and other tissues, including erythrocytes

b. **Clinical significance**

1) **AST** is used to evaluate **hepatocellular disorders** (up to 100 times upper reference limit in viral hepatitis, up to 20 times upper reference limit in infectious mononucleosis, and up to 4 times upper reference limit in cirrhosis), skeletal muscle disorders (up to 8 times upper reference limit), and pulmonary emboli (up to 3 times upper reference limit) and acute pancreatitis.

2) In **AMI, AST rises** within 6–8 hours, peaks at 18–24 hours, and returns to normal within 4–5 days. **AST** is **not used to diagnose AMI,** but awareness of the AST pattern may be **useful when ruling out other disorders, including concurrent liver damage.**

c. **Test methodology**

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST (P-5'-P)}} \text{oxaloacetate} + \text{glutamate}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{\text{malate dehydrogenase}} \text{malate} + NAD^+$$

1) **Sources of error:** Hemolysis

2) **Reference range:** 5–30 U/L at 37°C

5. **Alanine aminotransferase (ALT)**
   a. **Tissue location:** Highest concentrations in liver, with lesser amounts in other tissues, including kidneys and erythrocytes
   b. **Clinical significance:** Hepatocellular disorders (hepatitis, cirrhosis) exhibit higher ALT levels than intra- or extrahepatic obstruction. **ALT is more specific for liver disease** than AST. ALT, in conjunction with an elevated AST, is used to assess liver involvement with diagnosis of an AMI. ALT does not exhibit a significant increase in muscular dystrophy, and it is not affected in cases of pulmonary emboli or acute pancreatitis.
   c. **Test methodology**

$$\text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT (P-5'-P)}} \text{pyruvate} + \text{glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LD}} \text{lactate} + \text{NAD}^+$$

      1) **Sources of error:** Slight hemolysis does not interfere.
      2) **Reference range:** 6–37 U/L at 37°C

6. **Alkaline phosphatase (ALP)**
   a. **Tissue location:** Highest concentrations are found in liver, bone, intestines, spleen, kidney, and placenta. ALP is found on cell surfaces, in sinusoidal and bile canalicular membranes in the liver, and in bone osteoblasts. In normal adult serum, ALP is mainly of liver origin, with a small amount from bone.
   b. **Clinical significance**
      1) **Increased** serum ALP levels are seen in hepatobiliary disease and bone disorders (with osteoblastic involvement). In hepatobiliary disorders, the increased levels are due to **obstructive disease,** and the **ALP** levels are **increased more significantly than ALT and AST.**
         a) **In biliary tract obstruction,** synthesis of ALP is induced by cholestasis, which causes serum ALP levels to rise 3 to 10 times the upper reference limit. The elevation is usually greater in cases of extrahepatic obstruction in contrast to intrahepatic obstruction.
         b) In hepatitis and cirrhosis, which are classified as hepatocellular conditions, ALP rises up to 3 times the upper reference limit.
         c) Highest elevations of ALP are seen in Paget disease.
         d) ALP levels increase with healing bone fractures.
      2) **Decreased serum** ALP levels are seen in **hypophosphatasia** because of lack of ALP bone isoenzyme. This disorder is characterized by insufficient bone calcification.
      3) ALP levels are **normally higher in children** than adults because of bone growth.

4) ALP levels are **normally higher in** women during **pregnancy** because the placenta is a source of ALP.

c. **Test methodology**

$$\underset{\text{(colorless)}}{p\text{-Nitrophenylphosphate}} + H_2O \xrightarrow{\text{ALP (pH 10.2)}} \underset{\text{(yellow)}}{p\text{-nitrophenol}} + \text{phosphate ion}$$

1) **Sources of error:** Hemolysis
2) **Reference ranges:**
Adults: 50–115 U/L at 37°C
Children aged 4–15 years: 54–369 U/L at 37°C

7. **Acid phosphatase (ACP)**
   a. **Tissue location:** Highest concentration in **prostate gland,** with lesser amounts in bone (osteoclasts), liver, spleen, erythrocytes, platelets
   b. **Clinical significance**
      1) **Increased** in prostate cancer, benign prostatic hypertrophy, bone disease, Paget disease, breast cancer with bone metastases, Gaucher disease, platelet damage, idiopathic thrombocytopenic purpura
      2) ACP is **useful in forensic cases** involving rape because vaginal washings containing seminal fluid would exhibit ACP activity.
   c. **Test methodology**

$$p\text{-Nitrophenylphosphate} \xrightarrow{\text{ACP (pH 4.9)}} p\text{-nitrophenol} + \text{NaOH} \xrightarrow{\text{(pH 11.0)}} \text{quinoid form (410 nm)}$$

Prostatic ACP = Total ACP − ACP after tartrate inhibition

1) **Sources of error:** Hemolysis; loss of activity in 1–2 hours at room temperature
2) **Reference ranges:**
Total ACP: male 2.5–11.7 U/L; female 0.3–9.2 U/L at 37°C
Prostatic ACP: male 0.2–5.0 U/L; female 0.0–0.8 U/L at 37°C

8. **Gamma-glutamyltransferase (GGT)**
   a. **Tissue location:** GGT is found in liver (canaliculi of hepatic cells and epithelial cells lining biliary ductules), kidneys, pancreas, intestine, and many other tissues. GGT is **not** found in skeletal muscle tissue or bone.
   b. **Clinical significance**
      1) **Increased** levels in all **hepatobiliary** diseases, with levels increasing to 2–5 times the upper reference limit (e.g., viral hepatitis, alcoholic cirrhosis); very sensitive indicator for these conditions
      2) Higher levels observed in **intra- and posthepatic biliary tract obstruction,** with levels increasing to 5–30 times the upper reference

limit; increases before and remains elevated longer than ALP, AST, ALT

3) GGT activity **induced by drugs** (e.g., phenobarbital and phenytoin) and by alcohol consumption
4) GGT levels are **normal in** the presence of **bone disease** and during **pregnancy** in contrast to alkaline phosphatase, where levels would be elevated.

c. **Test methodology**

$$\underset{\text{(colorless)}}{\gamma\text{-Glutamyl-}p\text{-nitroanilide}} + \text{glycylglycine} \xrightarrow{\text{GGT}} \gamma\text{-glutamyl-glycylglycine} + \underset{\text{(yellow)}}{p\text{-nitroaniline}}$$

1) **Sources of error:** Hemolysis does not interfere.
2) **Reference ranges:** Male, up to 55 U/L; female, up to 38 U/L at 37°C

9. **Amylase (AMS)**
   a. **Tissue location:** Found in pancreas, salivary glands, small intestine, fallopian tubes, and other tissues
   b. **Clinical significance**
      1) **Increased** serum levels in **acute pancreatitis** occur in 2–12 hours after the onset of pain, with peak values in 24 hours, and return to normal in 3–4 days.
      2) **Increased:** Mumps, perforated peptic ulcer, intestinal obstruction, cholecystitis, ruptured ectopic pregnancy, mesenteric infarction, acute appendicitis
   c. **Test methodology**
      1) **Amyloclastic:** Measures decrease in starch substrate
      2) **Saccharogenic:** Measures formation of the product produced from starch (maltose)
      3) **Chromogenic:** Measures the formation of soluble starch fragments coupled with a chromogenic dye
      4) **Enzymatic:** Defined substrate used in coupled-enzymatic reactions
         a) **Sources of error:** In hyperlipidemia, triglycerides suppress AMS activity; morphine and other opiates falsely elevate AMS levels
         b) **Reference range:** 28–100 U/L at 37°C
10. **Lipase (LPS)**
   a. **Tissue location:** Found in pancreas, with lesser amounts in gastric mucosa, intestinal mucosa, adipose tissue
   b. **Clinical significance:**
      1) **Increased** serum levels in **acute pancreatitis** occur in 4–8 hours after the onset of pain, with peak values in 24 hours, and return to normal in 8–14 days.

2) **Increased:** Perforated peptic ulcer, duodenal ulcers, intestinal obstruction, cholecystitis

c. **Test methodology**

$$\text{Oleic acid emulsion (0.8\%)} + H_2O \xrightarrow{\text{LPS}} \text{fatty acids}$$

(measure decreased turbidity)

1) **Sources of error:** Hemolysis because hemoglobin inhibits LPS activity
2) **Reference range:** Up to 38 U/L at 37°C

11. **Cholinesterase**

a. Two related enzymes: **Acetylcholinesterase (AChE)/true cholinesterase** and **acylcholine acylhydrolase (PChE)/pseudocholinesterase**

b. **Tissue location**

1) **True cholinesterase** found in **red blood cells,** lungs, spleen, nerve endings, gray matter of brain
2) **Pseudocholinesterase** found in liver, pancreas, heart, white matter of brain, **serum**

c. **Clinical significance**

1) **Pseudocholinesterase** found in serum in decreased amount in hepatocellular disease due to decreased synthesis, e.g., hepatitis, cirrhosis
2) Decreased PChE occurs in **insecticide poisonings.**
3) PChE testing identifies individuals with atypical forms who are **at risk** of prolonged response to muscle relaxants used in surgery.

d. **Test methodology**

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{PChE}} \text{thiocholine} + \text{butyric acid}$$

$$\text{Thiocholine} + \text{5,5′-dithiobis-(2-nitrobenzoic acid)} \longrightarrow \text{5-mercapto-2-nitrobenzoic acid} + \text{mixed disulfide}$$

1) **Sources of error:** Hemolysis
2) **Reference ranges (PChE serum):** Male, 40–78 U/L; female, 33–76 U/L at 37°C

12. **Glucose-6-phosphate dehydrogenase (G6PD)**

a. **Tissue location:** Found in **erythrocytes,** adrenal glands, thymus, lymph nodes, spleen

b. **Clinical significance**

1) **Decreased:** Primary importance of **G6PD** is in cases of **deficiency,** inherited as a sex-linked trait (X-chromosome). In G6DP deficiency, a drug-induced hemolytic anemia occurs when an individual is

administered antimalarial drugs or primaquine. Hemolysis may also be caused by infections and after ingestion of fava beans.

2) **Increased:** Megaloblastic anemias and AMI

c. **Test methodology**

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PD}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

1) **G6PD deficiency** requires the analysis of a **red blood cell hemolysate.**
2) Analysis of **G6PD elevations** requires a **serum** sample.
3) **Reference range (RBC):** 8–14 U/g Hgb

G. Cardiac Profile

1. Upon arrival to the emergency department, a cardiac profile would be ordered to establish **baseline values.** Then the cardiac profile would be ordered for several samplings in **3- to 8-hour intervals** over a 12- to 24-hour period. Frequently blood is drawn every 3 hours for analysis during the first 12-hour period. Laboratory testing used to assess AMI includes **cardiac troponin T or I, CK-MB, and sometimes myoglobin.** In many institutions, once the cardiac troponin appears elevated, additional sampling and testing is halted and the elevated cardiac troponin is considered diagnostic for AMI.
2. **Troponin**
   a. **Tissue location:** Troponins T, I, and C form a complex of three proteins that bind to filaments of skeletal muscle and cardiac muscle to regulate muscle contraction.
   b. **Clinical significance**
      1) **cTnT or cTnI** (cardiac troponin T or cardiac troponin I) is used as an **AMI indicator** because of specificity and early rise in serum concentration following AMI.
      2) **In cases of AMI, cTnT** increases in 3–4 hours following infarction, peaks in 10–24 hours, and returns to normal in 10–14 days. **cTnI** increases in 3–6 hours following infarction, peaks in 14–20 hours, and returns to normal in 5–10 days.
   c. **Test methodology**
      1) Quantified by immunoassay
      2) **Reference ranges:** cTnT <0.03 ng/mL; cTnI <0.40 ng/mL (Values vary considerably among laboratories and are dependent on the methodology employed.)
3. **Myoglobin**
   a. **Tissue location:** Found in skeletal and cardiac muscles
   b. **Clinical significance**
      1) **Increased** in skeletal muscle injuries, muscular dystrophy, and AMI
      2) Myoglobin is released early **in cases of AMI,** rising in 1–3 hours and peaking in 5–12 hours, and returns to normal in 18–30 hours. However,

it is **not tissue specific.** It is better used as a negative predictor in the first 2–4 hours following chest pain.

c. **Test methodology**
   1) Quantified by immunoassay
   2) **Reference ranges:** Male, 30–90 ng/mL; female, <50 ng/mL

4. **Creatine kinase and CK-MB** previously discussed in section VI. ENZYMES AND CARDIAC ASSESSMENT, pages 43–44.

H. Natriuretic Peptides: Polypeptide Hormones

1. **Tissue location and function**
   a. Three forms: ANP, CNP, BNP
   b. Although effects are minimal, they function to **promote excretion of sodium and water** by increasing the glomerular filtration rate and decreasing the tubular reabsorption of sodium by the kidneys.
   c. **B-type (brain) natriuretic peptide (BNP)** is synthesized in and secreted from myocardial ventricles in response to ventricular volume expansion and pressure overload. BNP causes dilation of blood vessels and promotes sodium and water loss, thus reducing fluid load on the heart to improve cardiac function.
2. **Clinical significance:** BNP **increased** in **congestive heart failure** (CHF)
3. **Test methodology**
   a. BNP quantified by fluorescence and chemiluminescence immunoassays
   b. **Reference range:** BNP <100 pg/mL
4. **ProBNP** assay measures N-terminal proBNP **(NT-proBNP),** which is released when BNP is cleaved from precursor proBNP.
   a. NT-proBNP has a longer half-life than BNP.
   b. Measurement of NT-proBNP shows no interference from nesiritide (human recombinant BNP) administration to treat CHF.
   c. NT-proBNP is measured by electrochemiluminescence.

I. High-sensitivity CRP (hs-CRP)

1. C-reactive protein (CRP): β-globulin that is an acute-phase reactant
2. **High-sensitivity CRP** refers to the sensitivity of the assay to determine low levels in serum.
3. **Clinical significance:** Used as a **predictor for cardiovascular risk;** increased levels seen in inflammation, infection, stress, trauma, and AMI
4. **Test methodology**
   a. Quantified by immunoassay; hs-CRP detection limit 0.05 mg/L
   b. **Reference ranges:** Males, 0.3–8.6 mg/L; females, 0.2–9.1 mg/L
   c. **Cardiovascular risk classification:**

Low risk <1.0 mg/L; average risk 1.0–3.0 mg/L; high risk >3.0 mg/L

J. Homocysteine

1. **Clinical significance:** Elevated levels cause damage to arterial walls that precedes formation of plaques. It is an indicator of arterial inflammation.
2. **Test methodology**
   a. Immunoassay, fluorometric, chromatographic
   b. **Reference range:** 5–15 μmol/L

VII. LIVER FUNCTION AND PORPHYRIN FORMATION

A. Liver Function: Synthesis, Excretory, and Detoxification

1. **Synthesis:** Liver synthesizes proteins, coagulation factors, ammonia, carbohydrates, fat, ketones, vitamin A, enzymes, and so on.
2. **Bilirubin:** Principal pigment in bile that is derived from hemoglobin breakdown
   a. Bilirubin is produced in the reticuloendothelial system from the breakdown of hemoglobin from senescent red blood cells (RBCs). **Bilirubin** forms a **complex with albumin** for transport to the liver. In this form, bilirubin is **unconjugated** and **not** water soluble.
   b. **Bilirubin** is **conjugated** in the hepatocyte endoplasmic reticulum **with glucuronic acid to form bilirubin diglucuronide** (conjugated bilirubin). The reaction is catalyzed by **uridine diphosphate (UDP) glycuronyltransferase.** Conjugated bilirubin is **water soluble.** Conjugated bilirubin is excreted into the bile for storage in the gallbladder, secreted into the duodenum in response to gallbladder stimulation, and reduced by anaerobic bacteria in the intestine to **urobilinogen.** Some intestinal urobilinogen is reabsorbed; a portion returns to the liver and some enters the circulation for excretion in the urine, whereas the remaining portion in the intestines is oxidized by anaerobic bacteria for excretion in the stool as **urobilin.** Urobilin is an **orange-brown pigment** that gives stool its characteristic color.
   c. **Jaundice (icterus)** is a yellow discoloration that occurs when the bilirubin concentration in the blood rises (**>2–3 mg/dL**) and the bilirubin is deposited in the skin and sclera of the eyes.
   d. **Kernicterus:** Elevated bilirubin deposits in **brain tissue of infants,** affecting the central nervous system and resulting in mental retardation.
3. **Liver secretes bile** to assist in digestion. Bile salts are composed of cholic acid and chenodeoxycholic acid conjugated with glycine or taurine. **Bile** is **stored** in the **gallbladder.**
4. **Detoxification and drug metabolism:** The **liver** is the primary site in the body for synthesis of waste products (e.g., urea), conjugation of hormones and bilirubin to water-soluble forms, and conversion of drugs to metabolites for excretion in urine or stool.

B. Classification of Causes of Jaundice

1. **Prehepatic jaundice occurs** when there is **excessive erythrocyte destruction,** as seen in hemolytic anemias, spherocytosis, toxic conditions, hemolytic disease of the newborn caused by Rh or ABO incompatibility, and so on. In these cases, the rate of hemolysis exceeds the liver's ability to take up the bilirubin for conjugation. Prehepatic jaundice is characterized by an **increased level of unconjugated bilirubin** in the serum.
2. **Hepatic jaundice** occurs when the **liver cells malfunction** and cannot take up, conjugate, or secrete bilirubin.
   a. **Gilbert syndrome:** Defect in the ability of hepatocytes to take up bilirubin; due to **transport problem** of bilirubin from the sinusoidal membrane to the microsomal region; characterized by **mild increase** in serum level of **unconjugated bilirubin** (1.5–3.0 mg/dL)
   b. **Crigler-Najjar disease:** Partial or complete deficiency of **UDP-glycuronyltransferase;** little, if any, conjugated bilirubin formed, which causes **increased** serum level of **unconjugated bilirubin** (moderate to extremely elevated)
   c. **Dubin-Johnson syndrome:** Defective liver cell excretion of bilirubin due to **impaired transport in the hepatocyte** of conjugated bilirubin from microsomal region to the bile canaliculi; characterized by **increased** serum level of **conjugated bilirubin** with mild increase in unconjugated bilirubin
   d. **Neonatal physiological jaundice:** Level of **UDP-glycuronyltransferase** is **low at birth;** takes several days for the liver to synthesize an adequate amount of the enzyme to catalyze bilirubin conjugation; causes **increased** serum level of **unconjugated bilirubin**
   e. **Intrahepatic cholestasis:** May be caused by hepatocyte injury such as cirrhosis, bile duct injury such as Rotor syndrome, or neoplasms
3. **Posthepatic jaundice** occurs when an **obstruction blocks the flow of bile into the intestines.** This is referred to as **extrahepatic cholestasis** and may be caused by gallstones obstructing the common bile duct, neoplasms such as carcinoma of the ampulla of Vater or carcinoma of the pancreas, and inflammatory conditions such as acute cholangitis or acute pancreatitis. Posthepatic jaundice is characterized by a **significantly increased** level of **conjugated bilirubin** in serum, increased level of unconjugated bilirubin in serum, increased conjugated bilirubin in the urine, decreased urine and fecal urobilinogen, and **stool** that appears **pale in color.**

C. Other Disorders of the Liver

1. **Cirrhosis:** Result of **chronic scarring** of liver tissue turning it into nodules; may be caused by excessive alcohol ingestion over a long period of time, hemochromatosis, complication of hepatitis

2. **Tumors**
   a. Hepatocellular carcinoma or hepatoma: primary cancer of the liver
   b. Metastatic liver tumors: Arise from other cancerous tissue where the primary site was of lung, pancreas, gastrointestinal tract, or ovary origin
3. **Reye syndrome**
   a. **Cause is unknown,** but the **symptoms include** encephalopathy, neurologic abnormalities including seizures or coma, and abnormal liver function tests due to hepatic destruction.
   b. Occurs mainly in children, usually after a viral infection (varicella or influenza) and aspirin therapy
4. **Drug-related disorders:** Drugs, including phenothiazines, antibiotics, antineoplastic drugs, and anti-inflammatory drugs such as acetaminophen, may cause liver damage.
5. Acute and chronic **hepatitis**

D. Serum Enzymes Used to Assess Liver Function

1. Markers for **hepatocellular necrosis**
   a. **ALT:** Most specific for hepatocyte injury
   b. **AST:** Less specific than ALT; significant presence in other tissues
   c. **LD:** Least specific; significant presence in other tissues
2. Markers that reflect **cholestasis**
   a. **Alkaline phosphatase**
   b. **Gamma-glutamyl transferase**
3. Other tests to assess liver disorders
   a. Total bilirubin, direct bilirubin (conjugated), indirect bilirubin (unconjugated)
   b. Albumin
   c. Ammonia
   d. AFP

E. Test Methodology for Bilirubin

1. **Jendrassik-Grof total bilirubin test**

Bilirubin + sodium acetate + caffeine-sodium benzoate + diazotized sulfanilic acid → purple azobilirubin + alkaline tartrate → green-blue azobilirubin (600 nm)

2. **Direct spectrophotometric:** For newborns, bilirubin concentration is read directly by spectrophotometry and concentration is proportional to absorbance at 455 nm.
3. **Sources of error:** Hemolysis, lipemia; avoid exposure to sunlight and fluorescent lighting
4. **Reference ranges**
   Infants: Total bilirubin 2–6 mg/dL (0–1 day, full term)

Adults: Total bilirubin 0.2–1.0 mg/dL
Indirect bilirubin 0.2–0.8 mg/dL
Direct bilirubin 0.0–0.2 mg/dL

F. Test Methodology for Urobilinogen

1. Urobilinogen is the collective term for stercobilinogen, mesobilinogen, and urobilinogen.
2. **Urine urobilinogen assay**

   urobilinogen + *p*-dimethyl aminobenzaldehyde → red colored complex

3. **Sources of error:** Oxidation will occur if urine is allowed to stand; other compounds react, such as porphobilinogen
4. **Clinical significance (see Table 1-5■)**
   a. In **posthepatic obstruction, urobilinogen** formation is **decreased** because of impaired bilirubin excretion into the intestines. This is evidenced by a clay-colored (partial biliary obstruction) or chalky white stool (complete biliary obstruction).
   b. **Increased** urine urobilinogen is associated with hemolytic disease and hepatocellular disease, such as hepatitis.
5. **Reference range urine urobilinogen:** 0.1–1.0 Ehrlich units/2 hr

TABLE 1-5 CAUSES OF JAUNDICE

	Reference Range	Hemolytic Jaundice	Intrahepatic Early Hepatitis	Extrahepatic Obstructive
Serum Conj. Bilirubin	0.0–0.2 mg/dL	Normal or sl. ↑	↑	↑↑
Serum Unconj. Bilirubin	0.2–0.8 mg/dL	↑	↑↑	↑
Feces Urobilinogen	75–400 EU/d or (+2)	↑(+4)	↓(+1)	↓ or Neg
Urine Urobilinogen	0.5–4.0 EU/d or (+1)	↑(+4)	↑	↓ or Neg
Urine Bilirubin	Negative (Neg)	Neg	↑	↑

G. Porphyrin Formation

1. Heme is derived from a series of biochemical reactions that begin with the formation of aminolevulinic acid (ALA) from succinyl coenzyme A and glycine.

Through a second condensation reaction, two molecules of ALA condense and cyclize to form porphobilinogen (PBG). Because porphobilinogen is a monopyrrole, four molecules of porphobilinogen condense and cyclize to form the various porphyrinogens. Specific enzymes catalyze the formation of **uroporphyrinogen, coproporphyrinogen, protoporphyrinogen,** and protoporphyrin IX. Protoporphyrinogen IX is the immediate precursor of protoporphyrin IX.

   a. **Deficiency** of any of the specific **enzymes** that catalyze the formation of the porphyrinogens results in **excess formation** of the corresponding **porphyrin.** The enzyme deficiencies may be inherited or acquired.
   b. Protoporphyrin IX chelates iron to form heme.

2. The **porphyrins** that are of clinical significance include **uroporphyrin, coproporphyrin,** and **protoporphyrin.** Types of **porphyrias** include:
   a. Plumboporphyria
   b. Acute intermittent porphyria
   c. Congenital erythropoietic porphyria
   d. Porphyria cutanea tarda
   e. Hepatoerythropoietic porphyria
   f. Hereditary coproporphyria
   g. Variegate porphyria
   h. Erythropoietic porphyria
3. General characteristics of the porphyrias
   a. Overproduction or accumulation of porphyrins and precursors, such as porphobilinogen, in the **bone marrow** is termed **erythropoietic porphyrias** and in the **liver** it is termed **hepatic porphyrias.**
   b. Excess of early precursors, such as ALA and PBG, causes **neuropsychiatric** symptoms, including abdominal pain, vomiting, constipation, tachycardia, hypertension, and psychiatric symptoms.
   c. Excess of later intermediates, uroporphyrins, coproporphyrins, and protoporphyrins, causes **cutaneous** symptoms including photosensitivity, blisters, excess facial hair, and hyperpigmentation. Photosensitivity results from the deposition of porphyrins in the skin.
   d. Excess of early precursors and later intermediates causes **neurocutaneous** symptoms.
4. **Methods:** For measurement of aminolevulinic acid, porphobilinogen, uroporphyrin, and coproporphyrin, a 24-hour urine specimen should be collected.
   a. Refrigerate urine during collection; store in brown bottle to protect light-sensitive compounds
   b. Porphobilinogen more stable under alkaline conditions and aminolevulinic acid more stable under acid conditions; sodium bicarbonate used as a compromise to maintain the pH near 7

c. **Watson-Schwartz test** employs ***p*-dimethylaminobenzaldehyde** reagent (also known as Ehrlich's aldehyde reagent) to form a red condensation product with **porphobilinogen.**
d. Porphyrin compounds may be detected in acid solution by irradiating the solution with long-wave ultraviolet light, which causes the **porphyrins** to **fluoresce.** The intense orange-red fluorescence of the porphyrins is due to the conjugated unsaturation of the tetrapyrrole ring structure.
e. Porphyrins may be differentiated and quantified using HPLC with a fluorescence detector system.

VIII. ELECTROLYTES AND OSMOLALITY

A. Osmolality

1. **Colligative properties** refer to the properties of a solution that are influenced by the number of molecules in solution, but not their individual composition. There are four types of colligative properties: **boiling point, freezing point, osmotic pressure,** and **vapor pressure.**
2. **Osmolality**
   a. **Osmolality** is the measure of the number of dissolved particles in solution expressed as **osmoles per kilogram of water.** Serum osmolality is expressed as **milliosmoles/kg;** the reference range for serum is 275–295 mOsm/kg.
   b. Osmolality is **regulated by** the **hypothalamus** through the sensation of thirst and the signaling to secrete antidiuretic hormone (ADH). When the osmolality of the blood is increased, two processes occur:
      1) Consuming more water will decrease the osmolality.
      2) Posterior pituitary secretion of ADH will cause renal reabsorption of water and decrease the osmolality.
   c. **Osmometry:** Method used to measure all particles (molecules and ions) in solution; measure of osmolality
   d. Two formulas used to **calculate estimated osmolality:**

$$\mathbf{1.86\,Na + glucose/18 + BUN/2.8 + 9 = mOsm/kg}$$

$$\mathbf{2(Na) + glucose/20 + BUN/3 = mOsm/kg}$$

      1) In healthy individuals, the **calculated osmolality** equals the **measured osmolality.**
      2) The **osmolal gap** represents the difference between the measured and calculated osmolality. The osmolal gap **should be <15.** An **osmolal gap can exist** for a variety of reasons, including excess production of β-hydroxybutyrate, ingestion of toxins such as ethylene glycol, or ingestion of an excessive amount of alcohol.

3. **Measuring osmolality**
   a. Measuring **serum** and **urine osmolality** is useful in assessing electrolyte disorders and acid-base status. Major molecules measured by serum osmolality include **sodium, chloride, glucose,** and **urea.**
   b. **Freezing point depression osmometry:** Particles in solution cause the freezing point of pure water to be decreased, with the decrease in temperature being directly proportional to the total number of particles present.
   c. **Vapor pressure depression osmometry:** Water evaporation is decreased when solute is present in water, which is indicated by an inverse relationship between the osmolality of the solution (amount of particles present) and the vapor pressure.

B. Electrolytes: Sodium, Potassium, Chloride, and Total Carbon Dioxide

1. **Electrolytes:** Charged ions found in intracellular fluid, extracellular fluid, and interstitial fluid
   a. **Cations** are **positively** charged ions. The **major cations** in the body are sodium, potassium, calcium, and magnesium.
   b. **Anions** are **negatively** charged ions. The **major anions** in the body are chloride, bicarbonate, phosphate, sulfate, organic acids, and protein.
   c. Clinically, when electrolytes are ordered on an individual, the **term "electrolytes"** is understood to mean the **measurement of serum sodium, potassium, chloride,** and **total carbon dioxide** (bicarbonate). The serum concentration of these four electrolytes is quantified using **ion-selective electrodes (ISEs).**
2. **Sodium (Na^+)**
   a. **Major cation of extracellular fluid**
   b. **Reference range:** 136–145 mmol/L
   c. Changes in sodium result in changes in plasma volume.
   d. **Largest** constituent of plasma osmolality
   e. Sodium is excreted in the urine when the renal threshold for serum sodium exceeds 110–130 mmol/L.
   f. **Clinical significance**
      1) **Hyponatremia** occurs when serum sodium level is <135 mmol/L.
         a) **Depletional hyponatremia** can be due to diuretics, hypoaldosteronism (Addison disease), diarrhea, or vomiting, and severe burns or trauma.
         b) **Dilutional hyponatremia** can be due to overhydration, syndrome of inappropriate antidiuretic hormone (SIADH), congestive heart failure, cirrhosis, and nephrotic syndrome.
      2) **Hypernatremia** occurs when serum sodium level is >150 mmol/L.
         a) Usually occurs when water is lost as through diarrhea, excessive sweating, or diabetes insipidus, and when sodium is retained as

through acute ingestion, hyperaldosteronism, or infusion of hypertonic solutions during dialysis

3. **Potassium (K^+)**
   a. **Major intracellular cation**
   b. **Reference range:** 3.4–5.0 mmol/L
   c. Because the concentration of potassium in red blood cells is higher than in serum, any level of hemolysis will falsely increase serum potassium results.
   d. **Clinical significance**
      1) **Hypokalemia** occurs when serum potassium level is <3.0 mmol/L.
         a) Results from decreased dietary intake, hyperaldosteronism, diuretics, vomiting, diarrhea, laxative abuse, and excess insulin which causes increased cellular uptake of potassium
      2) **Hyperkalemia** occurs when serum potassium level is >5.0 mmol/L.
         a) Results from increased intake, renal failure, hypoaldosteronism, metabolic acidosis, increased red blood cell lysis, leukemia, chemotherapy
4. **Chloride (Cl^-)**
   a. **Major anion of extracellular fluid**
   b. **Reference range:** 98–107 mmol/L
   c. Chloride levels change proportionally with sodium.
   d. **Clinical significance**
      1) **Hypochloremia** occurs when serum chloride level is <98 mmol/L.
         a) Results from excessive vomiting, use of diuretics, burns, aldosterone deficiency
      2) **Hyperchloremia** occurs when serum chloride level is >107 mmol/L.
         a) Results from prolonged diarrhea, renal tubular disease, dehydration, excess loss of bicarbonate
5. **Bicarbonate (HCO_3^-)**
   a. **Second largest anion fraction of extracellular fluid**
   b. **Reference range:** 22–29 mmol/L
   c. Clinically, the concentration of total carbon dioxide ($ctCO_2$) is measured because it is difficult to measure HCO_3^-. $ctCO_2$ is comprised primarily of HCO_3^- along with smaller amounts of H_2CO_3 (carbonic acid), carbamino-bound CO_2, and dissolved CO_2. HCO_3^- accounts for approximately 90% of measured $ctCO_2$.
   d. Bicarbonate is able to buffer excess H^+, making bicarbonate an important buffer system of blood.
   e. **Clinical significance**
      1) **Decreased $ctCO_2$** associated with metabolic acidosis, diabetic ketoacidosis, salicylate toxicity
      2) **Increased $ctCO_2$** associated with metabolic alkalosis, emphysema, severe vomiting

6. **Anion gap:** This is a mathematical formula used to demonstrate electroneutrality of body fluids. It represents the difference between cations and anions that are not actually measured analytically when serum "electrolytes" are quantified. The unmeasured cations include calcium and magnesium, whereas the unmeasured anions include phosphate, sulfate, organic acids, and protein.
   a. Two **calculation methods** commonly used:

$$Na^+ - (Cl^- + HCO_3^-) = \text{anion gap}$$

expected anion gap: 7–16 mmol/L

$$(Na^+ + K^+) - (Cl^- + HCO_3^-) = \text{anion gap}$$

expected anion gap: 10–20 mmol/L

   b. **Increased anion gap** can be caused by uremia, lactic acidosis, ketoacidosis, hypernatremia, and ingestion of methanol, ethylene glycol, or salicylate. It is also used as an assessment of instrument errors.
   c. **Decreased anion gap** can be caused by hypoalbuminemia and hypercalcemia.

C. Calcium

1. **Calcium** exists in plasma in three forms: 50% **free (ionized),** 40% **bound to protein,** 10% **bound to anions.** It is the **free form** of calcium that is **biologically active.**
2. Decreased free (ionized) calcium levels cause muscle spasms or uncontrolled muscle contractions called **tetany.**
3. **Regulation: Serum calcium** is controlled by parathyroid hormone, vitamin D, and calcitonin.
   a. **Parathyroid hormone (PTH)**
      1) A **decrease** in free (ionized) calcium **stimulates** the release of PTH by the parathyroid gland, and a **rise** in free calcium **terminates** PTH release.
      2) In bone, PTH **activates osteoclasts** to break down bone with the release of calcium.
      3) In the kidneys, PTH **increases tubular reabsorption** of calcium and **stimulates hydroxylation of vitamin D** to the active form.
   b. **Vitamin D (cholecalciferol)**
      1) Obtained by diet or exposure to sunlight
      2) Initially, vitamin D is transported to the liver, where it is hydroxylated but still inactive. Then the hydroxylated form is transported to the kidneys, where it is **converted to 1,25-dihydroxycholecalciferol,** the **active form** of the vitamin.
      3) **Calcium absorption** in the intestines is enhanced by vitamin D. In addition, PTH increases tubular reabsorption of calcium in the kidneys.

c. **Calcitonin**
   1) Released by the parafollicular cells of the thyroid gland when serum calcium level increases
   2) **Inhibits vitamin D and parathyroid hormone** activity, thus decreasing serum calcium
   3) Medullary carcinoma of the thyroid gland is a neoplasm of the parafollicular cells, resulting in elevated serum levels of calcitonin.

4. **Clinical significance**
   a. **Hypercalcemia** is caused by primary hyperparathyroidism, other endocrine disorders such as hypothyroidism and acute adrenal insufficiency, malignancy involving bone, and renal failure.
   b. **Hypocalcemia** is caused by hypoparathyroidism, hypoalbuminemia, chronic renal failure, magnesium deficiency, and vitamin D deficiency.
5. **Methods, interferences, reference range**
   a. **Methods used to measure total serum calcium:** Spectrophotometric (ortho-cresolphthalein complexone, arsenazo III dye), ISE (ion-specific electrode), atomic absorption (reference method)
      1) Spectrophotometric methods use metallochromic indicators that bind calcium causing a color change. These methods are easily automated.
      2) With ISE analysis, the specimen must be acidified to convert protein-bound and complexed calcium to the free form in order to measure total calcium.
   b. **Measure free (ionized) serum calcium:** Ion-specific electrode measures free form. Measurement is temperature sensitive, and generally analysis is performed at 37°C.
   c. **Sources of error:** Cannot use oxalate, citrate, or EDTA anticoagulants; interferences for spectrophotometric methods include hemolysis, icterus, and lipemia; interferences for ion-specific electrode methods include protein buildup on electrode and change in blood pH *in vitro* before analysis
   d. **Reference ranges**
      Total calcium (adults): 8.6–10.3 mg/dL
      Free calcium (adults): 4.6–5.3 mg/dL

D. Phosphorus

1. **Regulation**
   a. **Phosphate** in the blood is absorbed from dietary sources, released from cells, or released from bone. Regulation occurs by reabsorption or excretion by the **kidneys.**
   b. Most important regulatory hormone is **PTH,** which increases renal excretion of phosphate.
   c. **Vitamin D** regulates phosphate by causing intestinal absorption and renal reabsorption.
2. **Clinical significance**

a. **Hyperphosphatemia** is caused by renal failure, hypoparathyroidism, neoplastic diseases, lymphoblastic leukemia, and intense exercise.
b. **Hypophosphatemia** is caused by diabetic ketoacidosis, hyperparathyroidism, asthma, alcoholism, and malabsorption syndrome.

3. **Methods, interferences, reference range**
   a. Ammonium molybdate + phosphate ions $\rightarrow$ phosphomolybdate complex (colorless) read at 340 nm
   b. When aminonaphtholsulfonic acid is used to reduce the complex, a colored product is formed and read at 600–700 nm.

$$\text{Phosphomolybdenum} \xrightarrow{+\text{electrons}} \text{molybdenum blue}$$

   c. **Sources of error:** Hemolysis, lipemia, icterus; cannot use oxalate, citrate, or EDTA anticoagulants
   d. **Reference range (adults):** 2.5–4.5 mg/dL

E. Magnesium

1. **Magnesium** exists in plasma in three forms: 55% **free (ionized),** 30% **bound to protein,** 15% **complexed.** It is the **free form** of magnesium that is **biologically active.**
2. Regulation
   a. The magnesium level is regulated by the **kidneys** through reabsorption and excretion.
   b. **PTH enhances** reabsorption by the kidneys and intestinal absorption.
3. **Clinical significance**
   a. **Hypermagnesemia** is caused by renal failure and excess antacids.
   b. **Hypomagnesemia** is caused by gastrointestinal disorders; renal diseases; hyperparathyroidism (hypercalcemia); drugs (e.g., diuretic therapy, cardiac glycosides, cisplatin, cyclosporine); diabetes mellitus with glycosuria; and alcoholism due to dietary deficiency.
4. **Methods, interferences, reference range**
   a. **Methods used to measure total serum magnesium:** Calmagite, methylthymol blue, atomic absorption spectrophotometry (reference method)
   b. **Measure free (ionized) serum magnesium:** Ion-selective electrode
   c. **Sources of error:** Hemolysis; cannot use oxalate, citrate, or EDTA anticoagulants
   d. **Reference range (adults):** 1.7–2.4 mg/dL

F. Serum Iron and Total Iron-Binding Capacity

1. **Iron** is found in several locations in the body, including: component of hemoglobin and myoglobin, stored form **(ferritin and hemosiderin),** tissue

compartment (component of enzymes and coenzymes), and labile pool. Iron is transported in the blood by **transferrin.**

a. **Serum iron** exhibits **diurnal variation,** with values being highest in the morning.
b. **Transferrin** is **increased** in iron-deficiency disorders, and it is **decreased** in conditions of iron overload, hemochromatosis, and severe infections. Transferrin is measured directly by immunochemical methods. **Transferrin** has a **reference range** of 200–360 mg/dL.
c. **Ferritin reflects iron stores.** Ferritin **decreases early** in iron-deficiency disorders, making it a sensitive, early indicator of disease. It is **increased** in conditions of iron overload, hemochromatosis, and severe infections. Ferritin is an acute-phase protein measured directly by immunochemical methods. **Ferritin reference ranges** are 20–250 ng/mL for males and 10–120 ng/mL for females.

2. **Clinical significance (see Table 1-6■)**
   a. **Decreased serum iron** is associated with iron-deficiency anemia, malnutrition, blood loss, and chronic infection.
   b. **Increased serum iron** is associated with iron overdose, sideroblastic anemia, viral hepatitis, and hemochromatosis.
3. **Test methodology**
   a. **Total iron content (serum iron):** Measures serum Fe^{3+} bound to transferrin. An acid solution is used to release Fe^{3+} from transferrin, Fe^{3+} is reduced to Fe^{2+} by a reducing agent, Fe^{2+} is complexed with a chromogen reagent such as bathophenanthroline or ferrozine.
   b. **Total iron-binding capacity (TIBC):** Measures the quantity of iron bound to transferrin **if all** the **binding sites** on transferrin were **occupied** (i.e., saturated with iron). Fe^{3+} is added to serum to saturate transferrin. $MgCO_3$ is added to remove unbound Fe^{3+}. The mixture is centrifuged and the supernatant is used in the serum iron procedure.

TABLE 1-6 DISEASE STATES RELATED TO IRON METABOLISM

Test	Iron Deficiency	Malnutrition	Iron Overdose	Hemochromatosis
Serum Iron	Decreased	Decreased	Increased	Increased
% Saturation	Decreased	Varies	Increased	Increased
TIBC (indirect transferrin)	Increased	Decreased	Decreased	Decreased

c. **Percent transferrin saturation:** This is a calculated value that represents the amount of iron that transferrin is **capable** of binding.

Calculate using serum iron and TIBC:

$$\%\ \textbf{transferrin saturation} = \textbf{serum iron}\ (\mu g/dL) \div \textbf{TIBC}\ (\mu g/dL) \times 100\%$$

4. **Reference ranges**
Serum iron: 45–160 μg/dL
TIBC: 250–425 μg/dL
% Saturation: 15–55

IX. ACID-BASE METABOLISM

A. Major Buffer Systems

1. **Buffer:** System that can resist change in pH; composed of a weak acid or a weak base and its corresponding salt
2. Four buffer systems of clinical importance exist in whole blood:
   a. The **bicarbonate-carbonic acid buffer system** uses HCO_3^- and H_2CO_3 to minimize pH changes in plasma and erythrocytes. It is the most important buffer system in plasma.
   b. The **protein buffer system** uses plasma proteins to minimize pH changes in the blood.
   c. The **phosphate buffer system** uses HPO_4^{2-} and $H_2PO_4^-$ to minimize pH changes in plasma and erythrocytes.
   d. The **hemoglobin buffer system** uses the hemoglobin in red blood cells to minimize pH changes in the blood. It is the most important intracellular buffer.

B. Definitions

1. **Respiration:** Process to supply cells with oxygen for metabolic processes and remove the carbon dioxide produced during metabolism
2. **Partial pressure:** In a mixture of gases, partial pressure is the amount of pressure contributed by each gas to the total pressure exerted by the mixture.
3. **Acidemia** occurs when arterial blood pH <7.35.
4. **Alkalemia** occurs when arterial blood pH >7.45.
5. **Hypercapnia** is increased blood PCO_2.
6. **Hypocapnia** is decreased blood PCO_2.
7. **Partial pressure of carbon dioxide (PCO_2):** Measured in blood as mm Hg
8. **Concentration of dissolved carbon dioxide ($cdCO_2$):** Includes undissociated carbonic acid (H_2CO_3) and carbon dioxide dissolved in blood **(represented by PCO_2)**

9. **Concentration of total carbon dioxide (ctCO$_2$):** Includes bicarbonate (primary component), carbamino-bound CO_2, carbonic acid, and dissolved carbon dioxide

C. Acid-Base Balance

1. The **pH of plasma** is a function of two independent variables: the **partial pressure of carbon dioxide (PCO$_2$),** which is regulated by the **lungs** or (respiratory mechanism), and the concentration of **bicarbonate** (HCO_3^-), which is regulated by the **kidneys** (renal mechanism).
2. Carbon dioxide is transported as bicarbonate, carbamino compound (bound to serum proteins and hemoglobin), and dissolved carbon dioxide. Even though these forms transport the carbon dioxide, they also serve as buffers to maintain blood pH. Carbon dioxide, pH, and PCO$_2$ are related according to the **Henderson-Hasselbalch equation:**

$$\text{pH} = \text{pK}' + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$\text{pH} = 6.1 + \log \frac{c\text{HCO}_3^-}{c\text{dCO}_2}$$

$$\boldsymbol{c\text{dCO}_2 = P\text{CO}_2 \times \alpha \text{ (solubility coefficient of CO}_2\text{)}}$$

$$\textbf{where } \alpha = \textbf{0.03 mmol/L per mm Hg}$$

The average normal ratio of $\boldsymbol{c\text{HCO}_3^-}$ to $\boldsymbol{c\text{dCO}_2}$ is **20:1.** So any change in the bicarbonate concentration or the dissolved carbon dioxide concentration (includes H_2CO_3) would result in a change in blood pH. Because laboratories measure the concentration of total carbon dioxide (ctCO$_2$), this value is substituted for $c\text{HCO}_3^-$ in the equation. If $c\text{tCO}_2 = 24$ mmol/L and $P\text{CO}_2 =$ 40 mm Hg, then

$$\text{pH} = 6.1 + \log \frac{c\text{tCO}_2}{[P\text{CO}_2 \times \alpha]}$$

$$\text{pH} = 6.1 + \log \frac{[24 \text{ mmol/L}]}{[40 \text{ mm Hg} \times 0.03 \text{ mmol/L/mm Hg}]}$$

$$\text{pH} = 6.1 + \log \frac{[24\ ]}{[1.2]}$$

$$\text{pH} = 6.1 + \log \frac{[20\ ]}{[1]}$$

$$\text{pH} = 6.1 + 1.3 = 7.4$$

3. **Reference ranges for arterial blood gas analysis**

   pH: 7.35–7.45

   ***c*tCO$_2$:** 22–26 mmol/L

   ***P*CO$_2$:** 35–45 mm Hg

D. Acid-Base Disorders

1. Acid-base disorders are classified as metabolic acidosis, metabolic alkalosis, respiratory acidosis, and respiratory alkalosis.
   a. **Metabolic** acid-base disorders primarily involve **bicarbonate concentration.**
   b. **Respiratory** acid-base disorders primarily involve **dissolved carbon dioxide concentration.**
2. **Metabolic acidosis (nonrespiratory): Primary bicarbonate deficit**
   a. In **metabolic acidosis,** the **bicarbonate concentration decreases,** causing a decrease in the 20:1 ratio between $c\text{HCO}_3^-$ and $c\text{dCO}_2$, which results in a decrease in the blood pH.
   b. **Metabolic acidosis** may be **caused by** organic acid production or when ingestion exceeds the excretion rate. Disorders include diabetic ketoacidosis due to the production of acetoacetic acid and β-hydroxybutyric acid; lactic acidosis due to the production of lactic acid; poisonings such as salicylate, ethylene glycol, and methyl alcohol; reduced acid excretion due to renal failure or tubular acidosis; and loss of bicarbonate due to diarrhea or excessive renal excretion.
   c. **Laboratory findings in metabolic acidosis**
      1) $c\text{tCO}_2$ decreased
      2) $P\text{CO}_2$ normal
      3) pH decreased
   d. **Respiratory compensatory mechanism:** A decreased pH triggers **hyperventilation** that **lowers *P*CO$_2$** and results in an increase in pH. This increases the ratio between $c\text{HCO}_3^-$ and $c\text{dCO}_2$ to 20:1, which increases the blood pH.
   e. **Laboratory findings in compensation**
      1) $c\text{tCO}_2$ decreased
      2) $P\text{CO}_2$ decreased
      3) pH normal
3. **Metabolic (nonrespiratory) alkalosis: Primary bicarbonate excess**
   a. In **metabolic alkalosis,** the **bicarbonate concentration increases,** causing an increase in the 20:1 ratio between $c\text{HCO}_3^-$ and $c\text{dCO}_2$, which results in an increase in the blood pH.
   b. **Metabolic alkalosisis** may be **caused by** ingestion of excess base, decreased elimination of base, or loss of acidic fluids. Disorders include ingestion of

excess alkali (antacids); intravenous administration of bicarbonate; renal bicarbonate retention; prolonged diuretic use; loss of hydrochloric acid from the stomach after vomiting, intestinal obstruction, or gastric suction; glucocorticoid excess as in Cushing syndrome; and mineralocorticoid excess as in hyperaldosteronism.

c. **Laboratory findings in metabolic alkalosis**
   1) $c\text{tCO}_2$ increased
   2) $P\text{CO}_2$ normal
   3) pH increased

d. **Respiratory compensation mechanism:** The pH increase slows breathing **(hypoventilation),** thus **increasing the amount of CO_2 retained** by the lungs. This increased CO_2 retention causes an increase in H_2CO_3, which results in more dissolved CO_2 in the blood. The carbonic acid lowers the pH. This decreases the ratio between $c\text{HCO}_3^-$ and $c\text{dCO}_2$ to 20:1, which decreases the blood pH.

e. **Laboratory findings in compensation**
   1) $c\text{tCO}_2$ increased
   2) $P\text{CO}_2$ increased
   3) pH normal

4. **Respiratory acidosis: Primary $c\text{dCO}_2$ excess expressed as increase in $P\text{CO}_2$ (hypercapnia)**

a. Inability of a person to exhale CO_2 through the lungs **(hypoventilation)** causes an **increase of $P\text{CO}_2$.** The increased $P\text{CO}_2$ causes an increase in the concentration of dissolved carbon dioxide, which forms carbonic acid in the blood. This decreases the 20:1 ratio between $c\text{HCO}_3^-$ and $c\text{dCO}_2$, which decreases the blood pH.

b. **Respiratory acidosis** may be **caused by** chronic obstructive pulmonary disease, such as chronic bronchitis and emphysema, ingestion of narcotics and barbiturates, and severe infections of the central nervous system such as meningitis.

c. **Laboratory findings in respiratory acidosis**
   1) $c\text{tCO}_2$ normal
   2) $P\text{CO}_2$ increased
   3) pH decreased

d. **Renal compensatory mechanism:** The **kidneys** increase sodium-hydrogen exchange, ammonia formation, and bicarbonate retention. The **increased bicarbonate** concentration aids the return of the 20:1 ratio, which raises the blood pH.

e. **Laboratory findings in compensation**
   1) $c\text{tCO}_2$ increased
   2) $P\text{CO}_2$ increased
   3) pH normal

5. **Respiratory alkalosis: Primary cdCO$_2$ deficit expressed as decrease in PCO$_2$ (hypocapnia)**
   a. **Decreased PCO$_2$** results from an accelerated rate or depth of respiration, or a combination of both. Excessive exhalation of carbon dioxide **(hyperventilation) reduces the PCO$_2$,** causing a decrease in the concentration of dissolved carbon dioxide, which forms less carbonic acid in the blood (i.e., less hydrogen ions). This increases the 20:1 ratio between cHCO$_3^-$ and cdCO$_2$, which increases the blood pH.
   b. **Respiratory alkalosis** may be **caused by** hypoxia, anxiety, nervousness, excessive crying, pulmonary embolism, pneumonia, congestive heart failure, salicylate overdose, and so on.
   c. **Laboratory findings in respiratory alkalosis**
      1) ctCO$_2$ normal
      2) PCO$_2$ decreased
      3) pH increased
   d. The **renal compensatory mechanism** corrects respiratory alkalosis by **excreting bicarbonate.**
   e. **Laboratory findings in compensation**
      1) ctCO$_2$ decreased
      2) PCO$_2$ decreased
      3) pH normal

E. Oxygen Metabolism

1. **Oxygen** is **transported bound to hemoglobin** present in red blood cells and in a **physically dissolved** state.
   a. Three factors control oxygen transport: the PO$_2$, free diffusion of oxygen across the alveolar membrane, and affinity of hemoglobin for oxygen.
   b. Release of oxygen to the tissues is **facilitated by** an increase in H^+ concentration and PCO$_2$ levels at the tissue level.
   c. Under normal circumstances, the saturation of hemoglobin with oxygen is 95%. When the PO$_2$ is >110 mm Hg, greater than 98% of hemoglobin binds to oxygen.
   d. When a person's oxygen saturation falls below 95%, either the individual is not getting enough oxygen or does not have enough functional hemoglobin available to transport the oxygen.
   e. The amount of functional hemoglobin available in the blood can be altered due to decreased red blood cells or presence of nonfunctional hemoglobin (e.g., carboxyhemoglobin or cyanmethemoglobin).
2. **Clinical significance of PO$_2$ levels in blood**
   a. Increased values (>95%) are observed with supplemental oxygen.
   b. **Hypoxemia:** Causes include decreased pulmonary diffusion, decreased alveolar spaces due to resection or compression, and poor

ventilation/perfusion (due to obstructed airways—asthma, bronchitis, emphysema, foreign body, secretions)

X. ENDOCRINOLOGY

A. Hormones

1. **Hormones** are chemical compounds secreted into the blood that affect target tissues generally at a site distant from original production.
2. **General function**
   a. **Multiple hormones** can **affect one physiological function** (e.g., carbohydrate metabolism under the control of insulin, glucagon, growth hormone, cortisol, and epinephrine).
   b. **Single hormone** can **affect several organs** to produce different physiological effects (e.g., cortisol).
3. Three classes of hormones: **steroids, proteins** (peptides or glycoproteins), and **amines**
   a. **Steroid hormones**
      1) Synthesized by **adrenal glands, gonads, and placenta**
      2) Synthesized from **cholesterol** as needed, not stored, **lipid-soluble**
      3) **Need a carrier protein** to circulate in the blood
      4) Clinically significant hormones include **cortisol, aldosterone, testosterone, estrogen,** and **progesterone.**
      5) **Mechanism of action: Free hormone** is transported across cell membrane to interact with **intracellular receptor;** complex binds to chromatin, producing mRNA; mRNA initiates production of proteins that carry out the function attributed to the specific hormone.
      6) Hormone synthesis is regulated through **negative feedback** by another hormone (e.g., **cortisol/ACTH).**
   b. **Protein hormones**
      1) Synthesized by **anterior pituitary, placenta, pancreas, and parathyroid glands**
      2) Synthesized, then stored in the cell as secretory granules until needed
      3) Do **not** need carrier proteins to enter blood; **water soluble**
      4) Clinically significant hormones include **follicle-stimulating hormone** (FSH), **luteinizing hormone** (LH), **thyroid-stimulating hormone** (TSH), **human chorionic gonadotrophin** (hCG), **insulin, glucagon, parathyroid hormone, growth hormone,** and **prolactin.**
         a) Glycoprotein hormones, **FSH, LH, TSH,** and **hCG,** composed of alpha and beta chains; alpha chains identical and **beta** chains **unique** for each hormone
         b) Peptide hormones synthesized as prohormone, cleaved to produce circulating hormone (e.g., insulin)

5) **Mechanism of action:** Protein hormones interact with a **cell membrane receptor.** This activates a second messenger system and then cellular action.
6) Hormone synthesis is regulated through **change in analyte concentration** in serum (e.g., **insulin/glucose**) and **negative feedback** by another hormone (e.g., **testosterone/FSH).**

c. **Amine hormones**
1) Synthesized by **thyroid and adrenal glands**
2) Synthesized from **amino acids**
3) **Some** amine hormones **require a carrier protein** and others do not.
4) Clinically significant hormones include **epinephrine, norepinephrine, thyroxine,** and **triiodothyronine.**
5) **Mechanism of action: Epinephrine and norepinephrine** do not bind to carrier proteins and interact with the **receptor site on the cell membrane. Thyroxine and triiodothyronine** circulate bound to carrier proteins, with the **free hormone** being transported across the cell membrane to interact with the **intracellular receptor.**
6) Hormone synthesis is regulated by **nerve stimulation, another hormone** (e.g., thyroxine/TSH), and **negative feedback.**

4. Methods for quantifying hormones need to be **sensitive** because of the extremely low levels of hormones in the circulation. Some of the more commonly used methods include enzyme-multiplied immunoassay technique (EMIT), fluorescent immunoassay (FIA), fluorescent polarization immunoassay (FPIA), chemiluminescent immunoassay (CLIA), electrochemiluminescence immunoassay (Electro CLIA), and high-performance liquid chromatography (HPLC).

B. Hypothalamus: Overview and Clinical Significance

1. Hormones produced by the hypothalamus and their function:
a. **Corticotropin-releasing hormone** (CRH): Stimulates secretion of adrenocorticotropic hormone (ACTH)
b. **Gonadotropin-releasing hormone** (GnRH): Stimulates secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)
c. **Growth hormone-releasing hormone** (GHRH): Stimulates secretion of growth hormone (GH)
d. **Thyrotropin-releasing hormone** (TRH): Stimulates secretion of thyroid-stimulating hormone (TSH) and prolactin
e. **Dopamine:** Inhibits prolactin release
f. **Somatostatin:** Inhibits secretion of TSH and GH
2. **Supraoptic and paraventricular nuclei** of the **hypothalamus** produce **antidiuretic hormone** (ADH), also known as vasopressin, and **oxytocin.** These hormones are transported to the **posterior pituitary for storage.**

3. **Diseases:** Tumors, inflammatory or degenerative processes, and congenital disorders

C. Anterior Pituitary: Overview and Clinical Significance

1. Hormones secreted by the anterior pituitary include **ACTH, LH, FSH, TSH, GH, and prolactin.**
2. **Adrenocorticotropic hormone**
   a. Corticotropin-releasing hormone stimulates secretion of ACTH, which in turn stimulates synthesis of cortisol.
   b. **Increased cortisol** levels turn off secretion of ACTH and CRH.
   c. **Decreased cortisol** levels stimulate secretion of ACTH through negative feedback, which promotes cortisol synthesis.
   d. ACTH and cortisol exhibit **diurnal variation,** with **highest levels** in the **morning** and lowest levels in late afternoon to early evening.
3. **Growth hormone** (also known as somatotropin)
   a. Hypothalamus controls the release of growth hormone from the anterior pituitary with growth hormone-releasing hormone, which is stimulatory, and somatostatin, which is inhibitory.
   b. Direct effect on metabolism in numerous tissues: Antagonistic effect to insulin in relationship to glucose metabolism, stimulates gluconeogenesis in the liver, stimulates lipolysis, and promotes protein synthesis
   c. **Reference range:** Basal level 2–5 ng/mL
   d. Clinical significance
      1) **Increased levels** in childhood result in pituitary **gigantism** and in adulthood in **acromegaly** (enlarged feet, hands, and facial bones, impaired glucose tolerance, hypertension). Acromegaly is generally caused by a growth hormone-secreting pituitary tumor.
      2) **Decreased levels**
         a) Adults: Caused by pituitary adenomas or irradiation
         b) Children: May be familial or caused by a tumor, craniopharyngioma; results in **pituitary dwarfism**
4. **Prolactin:** Secreted by pituitary lactotroph cells and released upon stimulation from TRH; dopamine inhibits release
   a. **Function:** Initiates and maintains lactation; effects reproduction through ovarian and testicular steroidogenesis; affects the immune system
   b. **Reference ranges:** Male: 3.0–14.7 ng/mL; female: 3.8–23.0 ng/mL
   c. Clinical significance
      1) **Increased prolactin levels** may be caused by pituitary adenomas that produce prolactin, trauma, inflammation, chronic renal failure, and as a side effect of the administration of certain drugs (e.g., tricyclic antidepressants, phenothiazines, reserpine). Hyperprolactinemia results in hypogonadism.

2) **Decreased prolactin** levels may be caused by a tumor that compresses or replaces normal pituitary tissue. This is seen in panhypopituitarism, where there is loss of all anterior pituitary function.

5. **Follicle-stimulating hormone** will be discussed under "Ovaries: Overview and Clinical Significance" and "Testes: Overview and Clinical Significance."
6. **Luteinizing hormone** will be discussed under "Ovaries: Overview and Clinical Significance" and "Testes: Overview and Clinical Significance."
7. **Thyroid-stimulating hormone** will be discussed under "Thyroid: Overview and Clinical Significance."

D. Posterior Pituitary: Overview and Clinical Significance

1. **Posterior pituitary (neurohypophyseal system): Antidiuretic hormone (ADH), also known as vasopressin, and oxytocin** are hormones released by the posterior pituitary, but they are synthesized in the hypothalamus, where they form secretory granules for transport down the nerve axons to the posterior pituitary for storage. Upon stimulation, the hormones are secreted by the posterior pituitary.
2. **Antidiuretic hormone**
   a. **Function:** ADH controls water homeostasis by affecting the permeability of the collecting tubules of the kidney and enhancing water resorption, which makes the urine more concentrated and the blood more dilute. The osmolality of plasma has a regulatory effect on secretion of ADH. In addition, ADH raises blood pressure by stimulating musculature of arterioles and capillaries, affects uterine contraction, and promotes intestinal muscle contraction.
   b. Clinical significance
      1) **Increased ADH level** (hyperfunction): The **syndrome of inappropriate ADH secretion (SIADH)** occurs when there is uncontrolled secretion of ADH without any known stimulus for such release. In this syndrome, ADH is released even though the blood volume is normal or increased and plasma osmolality is low. This disorder may be caused by ectopic tumor production of ADH as in small cell carcinoma of the lung, central nervous system (CNS) disease, pulmonary disease, or as a side effect of administration of certain drugs.
      2) **Decreased ADH level** (hypofunction): Results in **polyuria,** causing **diabetes insipidus** and polydipsia
3. **Oxytocin**
   a. **Function:** Uterine stretch receptors stimulate the release of oxytocin, which in turn stimulates uterine contractions during childbirth. The action of suckling stimulates tactile receptors that promote the secretion of oxytocin, which causes ejection of breast milk.
   b. Although oxytocin is present in males, its function is unknown.

E. Adrenal Glands: Overview and Clinical Significance

1. **Adrenal glands:** Located above each kidney
   a. **Adrenal cortex (produces steroid hormones):** Outer portion of the gland, composed of three layers
      1) **Zona glomerulosa,** outermost layer, secretes **mineralocorticoids,** with **aldosterone** being the major hormone.
      2) **Zona fasciculata,** second layer, secretes **glucocorticoids,** with **cortisol** being the major hormone.
      3) **Zona reticularis,** third layer, secretes **sex hormones, principally the androgens.** Excessive production of androgens causes virilization.
   b. **Adrenal medulla (produces amine hormones):** Inner portion of the gland
      1) **Epinephrine** and **norepinephrine** are secreted and are known collectively as **catecholamines.**
2. **Steroid hormones** secreted by the adrenal glands are divided into three groups:
   a. **Mineralocorticoids:** Regulate salt balance
   b. **Glucocorticoids:** Assist with carbohydrate metabolism
   c. **Androgens:** Required for sexual function (contribution from the adrenal glands is minimal as compared to the gonads)
3. **Aldosterone** controls the retention of Na^+, Cl^-, and H_2O, the excretion of K^+ and H^+ and, therefore, the amount of fluid in the body.
   a. Aldosterone production is controlled by the **renin-angiotensin system** of the kidney. When the juxtaglomerular apparatus of the kidney detects low serum sodium or pressure changes in the blood perfusing the kidneys, due to decreased blood pressure or blood volume, **renin** is **produced.** Renin is a protein that acts on angiotensinogen to produce angiotensin I, which is acted on by angiotensin-converting enzyme to catalyze the formation of angiotensin II. **Angiotensin II** stimulates the **secretion of aldosterone** and is a **potent vasoconstrictor.**
   b. **Function** of aldosterone is to **increase salt and water conservation** through renal tubular retention of Na^+ and Cl^- and H_2O secondarily and to promote excretion of K^+ and H^+.
      1) Overall effect is vasoconstriction, which increases blood pressure (BP), and Na^+ retention, which promotes increase in blood volume (BV).
      2) Increase in BP and BV suppresses secretion of renin and, thus, the synthesis of aldosterone.
   c. **Reference ranges:** Adult supine, 3–16 ng/dL; adult upright, 7–30 ng/dL; blood levels of aldosterone are higher in the morning
   d. **Clinical significance**
      1) **Hyperaldosteronism**
         a) **Primary hyperaldosteronism:** Adrenal disease such as an aldosterone-secreting adrenal adenoma (Conn syndrome),

aldosterone-secreting adrenal carcinoma, or hyperplasia of adrenal cortex

b) **Secondary hyperaldosteronism:** Renin-angiotensin system disorder due to excess production of renin, malignant hypertension, or a renin-secreting renal tumor

2) **Hypoaldosteronism**

a) Atrophy of adrenal glands

b) Symptoms of **Addison disease:** Atrophy of adrenal glands with depressed production of aldosterone and the glucocorticoids

i) Hypoadrenalism causes decreased secretion of aldosterone and cortisol, increased ACTH, increased β-MSH, decreased blood glucose; decreased Na^+ and Cl^-, and increased K^+.

ii) Pigmentation of the skin, muscle weakness, weight loss, decreased blood pressure, nausea, diarrhea

c) **Congenital deficiency of 21-hydroxylase enzyme**

4. Cortisol

a. **Physiological effects of cortisol include** anti-insulin effects on carbohydrates that result in increased blood glucose levels, increased gluconeogenesis, increased lipolysis, increased protein catabolism, decreased protein synthesis, decreased antibody formation, and suppressed inflammatory response.

b. **Regulation of cortisol:** The hypothalamus secretes corticotropin-releasing hormone and the anterior pituitary secretes adrenocorticotropic hormone, which controls cortisol production via a feedback loop.

1) Low levels of plasma cortisol promote ACTH release.

2) Elevated levels of plasma cortisol inhibit ACTH release.

c. **Reference ranges total cortisol:** 8 A.M., 5–23 μg/dL; 4 P.M., 3–16 μg/dL; cortisol and ACTH exhibit **diurnal variation**

d. Clinical significance

1) **Hypercortisolism**

a) **Primary hypercortisolism:** Adrenal adenoma or carcinoma, exogenous administration of cortisol, Cushing syndrome (results from cortisol excess regardless of cause)

b) **Symptoms of Cushing syndrome**

i) Increased serum cortisol; cortisol lacks diurnal variation; hyperglycemia

ii) When adrenal gland secretes excess cortisol, the ACTH will be decreased.

iii) Weight gain in the face (moon face) and abdomen, buffalo hump back, thinning of skin, easy bruising, hypertension, muscle wasting, decreased immune response

c) **Secondary hypercortisolism:** Excessive production of ACTH due to pituitary tumor, ectopic production of ACTH by nonendocrine

tumor, Cushing disease (results from pituitary ACTH excess, which stimulates excess cortisol production)

2) **Hypocortisolism**
   a) **Primary hypocortisolism:** Atrophy of adrenal gland, autoimmune disease, tuberculosis, prolonged high-dosage cortisol therapy
   b) **Secondary hypocortisolism:** Pituitary hypofunction

5. **Adrenal medulla: Inner portion**
   a. Catecholamines synthesized from **tyrosine** by chromaffin cells of the adrenal medulla, brain, and sympathetic neurons
   b. **Catecholamines** include the hormones **epinephrine, norepinephrine,** and **dopamine.**
   c. **Function**
      1) **Epinephrine:** Mobilizes energy stores by converting glycogen to glucose, which allows the voluntary muscles to have greater work output; released in response to low blood pressure, hypoxia, cold exposure, muscle exertion, and pain
      2) **Norepinephrine:** Functions as a neurotransmitter affecting the vascular smooth muscle and heart; released primarily by the postganglionic sympathetic nerves
      3) **Dopamine:** Functions as a neurotransmitter in the brain affecting the vascular system
   d. Epinephrine and norepinephrine are **metabolized into metanephrine** and **normetanephrine** and then to final end product **vanillylmandelic acid** (VMA). Some metanephrine and normetanephrine along with the end product VMA are excreted in the urine.
   e. Increased levels of epinephrine and norepinephrine are associated with **pheochromocytoma** (tumor of the adrenal medulla, usually benign).
      1) Fluorometric methods used for quantifying plasma epinephrine and norepinephrine
      2) Colorimetric/spectrophotometric method used for quantifying VMA
   f. **Neuroblastoma** is a **malignant tumor** of the adrenal medulla that occurs in **children.** This tumor produces epinephrine and norepinephrine along with dopamine. The end product of dopamine metabolism is homovanillic acid (HVA).
      1) Characterized by increase in HVA and VMA urinary excretion
      2) May be quantified using HPLC, gas chromatographic and spectrophotometric methods

F. Ovaries: Overview and Clinical Significance

1. Ovaries are part of the **hypothalamic-pituitary-gonadal axis.**
   a. The anterior pituitary secretes **follicle-stimulating hormone,** which stimulates growth of the **ovarian follicles** and **increases** the **plasma**

estrogen level. FSH is under the control of gonadotropin-releasing hormone.

b. The anterior pituitary secretes **luteinizing hormone,** which stimulates production of **progesterone at ovulation.** LH is under the control of GnRH.

c. Estrogens and progesterone exert **negative feedback** to the hypothalamus and pituitary, which controls FSH and LH synthesis.

d. Abnormal synthesis of estrogens may be caused by the ovaries (primary disorder) or as a secondary disorder due to a primary disorder of the pituitary or hypothalamus.

2. **Estrogens and progesterone** are the principal **female sex hormones.**

a. **Estrogens** are secreted by the ovarian follicles and by the placenta in pregnancy (and to a much lesser extent by the adrenal glands and testes).

1) There are three primary estrogens: **estradiol-17β, estrone,** and **estradiol.**

2) **Estradiol** is the **principal estrogen** synthesized by the ovaries.

b. **Progesterone** is secreted by the ovarian follicles, mainly the corpus luteum following ovulation, and by the placenta in pregnancy.

c. **Function: Estrogen** promotes development and maintains the female reproductive system, including the uterus, fallopian tubes, and vagina. It is responsible for development and maintenance of secondary female sex characteristics (e.g., breast development, maturation of external genitalia, fat deposition, termination of bone growth). **Progesterone** is secreted by the corpus luteum following ovulation, and in pregnancy progesterone is secreted by the placenta to maintain the uterus.

d. Hormone changes in the **menstrual cycle**

1) In the first half of the menstrual cycle, **FSH** promotes growth of ovarian follicles and an increase in estrogen (low in first 7 days of cycle).

2) **Estrogen peaks at midcycle,** causing a decrease in FSH but promoting the **LH surge** at midcycle.

3) **LH triggers ovulation,** which is followed by a decrease in estrogen and LH levels.

4) The **follicle becomes** the **corpus luteum,** which **produces estrogen** and **progesterone.**

5) **Lack of fertilization** (thus absence of human chorionic gonadotropin) causes the **corpus luteum** to **degenerate** along with decrease in the estrogen and progesterone levels. **Progesterone falls** to the initial low level of the follicular phase about 24 hours prior to onset of menstruation.

6) Menstruation results, and then the cycle begins again.

7) Menstrual cycle

a) **Follicular phase** (first half): Characterized by **estrogen** stimulating growth of the uterine lining; progesterone levels are low

b) **Luteal phase** (second half): Characterized by **progesterone** promoting endometrium tissue to accept the fertilized ovum; progesterone measurements clinically useful to confirm ovulation

e. **Clinical significance**

1) **Hyperestrinism in females:**

a) **Precocious puberty:** Ovarian tumor, hypothalamic tumor, adrenal tumors (rare); may be difficult to determine

b) **Infertility and irregular menses:** Polycystic ovaries, estrogen-producing ovarian tumors, disorders of the hypothalamus or pituitary

c) **Postmenopausal bleeding:** Cervical or endometrial carcinoma, estrogen-producing ovarian tumors, exogenous estrogen consumption

2) **Hyperestrinism in males** results in testicular atrophy and enlargement of the breasts.

3) **Hypoestrinism**

a) **Ovarian insufficiency** can be primary or secondary to disorders of the hypothalamus or pituitary.

b) **Delayed puberty:** Primary amenorrhea due to lack of ovarian function or secondary to disorders of the hypothalamus or pituitary

c) **Amenorrhea** occurs at menopause, with radiation or chemotherapy, severe stress, intense athletic training, excessive weight loss.

d) **Turner syndrome** is a genetic defect in females where there is partial or complete loss of one of the two X chromosomes, resulting in nonfunctional ovaries. Exogenous estrogen can be administered to develop secondary sex characteristics.

4) **Hyperprogesteronemia:** Prevents menstrual cycle from occurring

5) **Hypoprogesteronemia:** Causes infertility, abortion of fetus

3. Estrogens in pregnancy

a. **Placenta** is the main source of estrogen synthesis during pregnancy, making primarily **estriol.**

b. Placenta requires a precursor compound that can **only** be made by the **fetal adrenal glands,** the **hydroxylated form of DHEAS** (16α-OH dehydroepiandrosteronesulfate); placenta **lacks** the enzyme 16α-hydroxylase.

c. Use **maternal estriol** blood level/urine excretion to **assess fetoplacental status.**

4. **Triple test** consists of **α_1-fetoprotein** (AFP), **unconjugated estriol** (uE3), and **human chorionic gonadotropin** (hCG).

a. Maternal blood sample collected at 16–18 weeks gestation

b. Triple test helps to **estimate risk of Down syndrome.** Following pattern is suggestive of increased risk:

1) **Decreased AFP** (made by fetal liver; found in maternal blood)

2) **Decreased uE3** (made by joint effort of fetus and mother)
3) **Increased hCG** (made by placenta)
4) Interpretation utilizes **MoMs: Multiples of the median**
5) Definitive testing would follow: Amniocentesis and chromosome analysis

5. **Quadruple (Quad) test** includes the analytes of the triple test **plus inhibin A,** a polypeptide hormone. **Inhibin A** would be **increased** in Down syndrome. In pregnancy, inhibin A is produced by the fetoplacental unit; function is to inhibit production of FSH.

G. Placenta: Overview and Clinical Significance

1. Placenta synthesizes and secretes **estrogens, progesterone, human chorionic gonadotropin,** and **human placental lactogen.**
2. **Human chorionic gonadotropin** prolongs the viability of the corpus luteum, which synthesizes progesterone and estrogens in early pregnancy until the placenta can assume the function. hCG levels are highest in the first trimester.
   a. **hCG qualitative** measurement used to **detect pregnancy.** Utilize monoclonal antibody to detect hCG in 1–2 days following fertilization.
   b. **hCG quantitative measurement**
      1) **Increased hCG:** Hydatidiform mole, choriocarcinoma, pre-eclamptic toxemia
      2) **Decreased hCG:** Threatened abortion, ectopic pregnancy
      3) hCG is used to monitor success of surgery and chemotherapy.
3. **Human placental lactogen** (HPL) functions with hCG to produce estrogen and progesterone during pregnancy. HPL level rises throughout gestation and reaches its highest level near term.
   a. HPL reflects integrity of placental function, so serial analysis may be helpful in high-risk pregnancies.
   b. **Decreased HPL** suggestive of placental malfunction and **potential fetal distress.**

H. Testes: Overview and Clinical Significance

1. Testes are part of the **hypothalamic-pituitary-gonadal axis.**
   a. The anterior pituitary secretes **follicle-stimulating hormone,** which stimulates **spermatogenesis.** FSH is under the control of GnRH.
   b. The anterior pituitary secretes **luteinizing hormone,** which stimulates production of **testosterone.** LH is under the control of GnRH.
   c. Through **negative feedback** to the hypothalamus, increased levels of testosterone shut off FSH and LH synthesis.
   d. Abnormal synthesis of testosterone may be caused by the testes (primary disorder) or as a secondary disorder due to a primary disorder of the pituitary or hypothalamus.
2. **Testosterone** is the principal **male sex hormone** and is secreted by the testes (and to a much lesser extent by the adrenal glands and ovaries).

a. **Function: Testosterone** promotes development and maintains the **male reproductive system.** It is responsible for development and maintenance of secondary male sex characteristics (e.g., facial and body hair, muscle development).
b. **Clinical significance**
   1) **Hyperandrogenemia:** In adult males, there are no observable symptoms. In prepubertal males, precocious puberty occurs (may be caused by hypothalamic tumors, congenital adrenal hyperplasia, testicular tumor). In female children, development of male secondary sex characteristics/virilization occurs (increased androgen production by ovaries or adrenals as androgens are estrogen precursors in females).
      a) **Congenital adrenal hyperplasia** (CAH) is caused by an enzyme defect of **21-hydroxylase,** which prevents cortisol production and results in accumulation of cortisol precursors, including 17-α-hydroxyprogesterone (17-OHP). CAH is characterized by **increased** blood levels of **17-OHP** and ACTH and decreased cortisol.
   2) **Hypoandrogenemia:** In adult males, impotence and loss of secondary sex characteristics occurs; in prepubertal males, delayed puberty results.
      a) **Primary hypoandrogenemia:** Causes include infections, tumors, congenital disorders (Klinefelter syndrome)
         i) **Klinefelter syndrome:** Male possesses an extra X chromosome (XXY). Characteristics include tall with long extremities, small testes, gynecomastia, infertility, and low IQ.
      b) **Secondary hypoandrogenemia:** Causes include primary hypofunction disorders of the pituitary or hypothalamus, which in turn cause decreased synthesis of LH and FSH

I. Thyroid Gland: Overview and Clinical Significance

1. **Thyroid gland** located in trachea-larynx area; composed of two lobes that consist of two types of cells
   a. **Follicular cells** are single layer of epithelial cells arranged spherically to create a follicle.
      1) **Make and secrete thyroid hormones**
         a) **T_4,** L-thyroxine
         b) **T_3,** L-triiodothyronine
         c) **rT_3,** reverse T_3 biologically inactive
      2) Hormones **stored** in lumina of follicle
   b. **Parafollicular cells** secrete **calcitonin,** which is involved with calcium regulation.
2. **Function:** Thyroid hormones aid in regulation of several metabolic functions, including rate of O_2 consumption and heat production, growth, sexual maturity, and protein and carbohydrate metabolism.

3. **Hypothalamic-pituitary-thyroid axis**
   a. **Thyrotrophin-releasing hormone** (TRH) is released by hypothalamus and stimulates anterior pituitary to secrete thyroid-stimulating hormone.
   b. **TSH** is a polypeptide hormone that originates in the anterior pituitary gland. TSH regulates synthesis and release of the thyroid hormones.
   c. Secretion of TSH is **regulated** by TRH, somatostatin, free T_3 (FT_3), and free T_4 (FT_4).
      1) **Somatostatin** functions as an inhibitory factor.
      2) FT_3 and FT_4 stimulate hypothalamus to secrete somatostatin.
      3) FT_3 and FT_4 exert negative feedback to the anterior pituitary to inhibit TSH secretion.
   d. It is estimated that 40% of secreted **T_4** undergoes enzymatic **monodeiodination** in tissues to **produce T_3** and approximately 45% is converted to **rT_3,** which is **biologically inactive.**
      1) **Thyroid hormones** circulate in blood **bound** to **thyroxine-binding globulin (TBG),** thyroxine-binding prealbumin, and thyroxine-binding albumin.
      2) **TBG is the principal carrier protein.**
      3) The **free hormones,** FT_3 and FT_4, are **physiologically active.**
      4) **T_3** is four to five times **more metabolically potent** in the tissues than T_4.
4. **Thyroid antibodies:** Appear with some autoimmune thyroid diseases
   a. **Thyroid-stimulating immunoglobulins** (TSI) are classified as **thyrotropin-receptor antibodies** (TRAbs). They **bind to TSH receptor sites** and activate thyroid epithelial cells.
   b. **Thyroid antimicrosomal antibodies** (TMAbs) cause tissue destruction, and analysis is generally directed to measurement of **antithyroid peroxidase antibodies** (TPOAbs). TPOAbs are detected in Hashimoto thyroiditis and in Graves disease.
   c. **Antithyroglobulin antibodies** (TgAbs) do not cause damage to the gland.
5. **Clinical significance (see Table 1-7■)**
   a. **Hypothyroidism** characterized by enlarged thyroid gland (goiter), impaired speech and memory, fatigue, weight gain, personality changes, cold intolerance, increased serum cholesterol and LDL, and so on.
      1) In **primary hypothyroidism,** total T_3 (TT_3), total T_4 (TT_4), FT_3, and FT_4 are decreased in the serum; TSH is increased in the serum.
      2) **Myxedema:** Advanced form of hypothyroidism
      3) **Congenital hypothyroidism/cretinism:** If untreated in first 3 months of life, irreversible neurological and mental deficiency occurs; newborn screening is required in U.S.
      4) **Hashimoto disease:** Most common cause of primary hypothyroidism; chronic autoimmune thyroiditis; TPOAb, TMAb, and TgAb present

TABLE 1-7 DISORDERS RELATED TO THE THYROID GLAND

Clinical Condition	TT_4	TT_3	FT_4	TSH
Primary Hypothyroidism	↓	↓	↓	↑
Secondary Hypothyroidism	↓	↓	↓	↓
Primary Hyperthyroidism	↑	↑	↑	↓
Secondary Hyperthyroidism	↑	↑	↑	↑
Primary Increase TBG	↑	↑	N	N
Primary Decrease TBG	↓	↓	N	N

↑ = Increased; ↓ = Decreased; N = Normal (within reference range)

5) **Hypothyroidism may be secondary or tertiary** to lack of TSH (pituitary disorder) or lack of TRH (hypothalamus disorder), respectively.

b. **Hyperthyroidism** is characterized by weight and muscle loss, fatigue, heat intolerance, nervousness, exophthalmos.
   1) In **primary hyperthyroidism,** total T_3, total T_4, FT_3, and FT_4 are increased in the serum; TSH is decreased in the serum.
   2) **Thyrotoxicosis:** Increased serum levels of thyroid hormones
   3) **Thyroid storm:** Life-threatening complication of uncontrolled thyrotoxicosis
   4) **Graves disease:** Most common cause of thyrotoxicosis; exhibits diffuse toxic goiter; autoimmune disorder with TRAb and TSI present
   5) **Hyperthyroidism may be secondary or tertiary** to increased levels of TSH (pituitary disorder) or increased levels of TRH (hypothalamus disorder), respectively.

6. Methods of measurement for **total thyroid hormone** and **TSH** include competitive immunoassays, enzyme immunoassays, chemiluminescence immunoassays; **direct measurement of free thyroid hormones** includes direct equilibrium dialysis and ultrafiltration methods, whereas **indirect methods for estimating free thyroid hormones** include two-step microparticle capture immunoassays and one-step immuno-chemiluminometric assays.
7. With availability of **highly sensitive TSH assays,** TSH testing is used to **screen for thyroid disorders** and to follow success of treatment protocols.

TSH reflects the physiological action of the thyroid hormones at the level of one of its target tissues, the pituitary gland. The secretion of TSH by the pituitary gland is very sensitive to changes and reflective of such changes in thyroid hormone concentration in the blood.

8. **Euthyroid** refers to a **normal functioning thyroid gland** in the presence of an abnormal concentration of **thyroxine-binding globulin** (TBG). A **primary increase** in the concentration of **TBG** is seen in conditions such as pregnancy and estrogen therapy. This manifests as an **increase in total T_4,** but the individual has **normal levels of FT_4 and TSH** due to negative feedback regulation to the anterior pituitary being intact. A **primary decrease** in the concentration of **TBG** is seen in conditions such as nephrotic syndrome, decreased protein production, and ingestion of certain drugs. This manifests as a **decrease in total T_4,** but the individual has **normal levels of FT_4 and TSH** due to negative feedback regulation to the anterior pituitary being intact.
9. An electrochemiluminescence immunoassay can be used for the **T uptake (TU)** test, which measures the **unsaturated serum binding capacity of TBG;** or, rephrased, TU measures available binding sites on TBG. **Thyroid hormone binding ratio (THBR)** expresses a ratio of T uptake in a patient's serum with a normal or reference serum.
   a. There is an **inverse** relationship between **THBR** levels and the **concentration of TBG.** When the serum concentration of TBG is increased (as in euthyroid primary increase in TBG), THBR is decreased.
   b. The **Free T_4 index (FT_4I)** is an indirect estimation of the free T_4 concentration in serum adjusted for any interference that may be caused by an abnormality in the binding proteins.

$$\textbf{Free } T_4 \textbf{ index } (FT_4I) = \textbf{Total } T_4 \times \textbf{ THBR}$$

J. Parathyroid Glands: Overview and Clinical Significance

1. **Four parathyroid glands** are located bilaterally on or near the thyroid gland capsule. Parathyroid glands are composed of chief cells and oxyphil cells. Chief cells synthesize, store, and secrete **parathyroid hormone** (PTH).
   a. PTH is synthesized as a **preprohormone.**
   b. **Amino N-terminal third is biologically active.**
   c. In the blood, intact PTH has half-life of **<5 minutes.**
2. **Function: PTH** aids in the **regulation of calcium** and **phosphate**, having direct action on bone and kidney and indirect action on the intestines through vitamin D. PTH increases the serum calcium level by increasing calcium resorption from bone, increasing calcium reabsorption in the renal tubules,

and increasing intestinal absorption of calcium by stimulating production of vitamin D.

   a. In **kidneys, PTH increases calcium reabsorption** in the distal tubule and decreases reabsorption of phosphate in the proximal tubule, resulting in phosphaturia.
   b. In **intestines, PTH promotes absorption of calcium** and phosphate by stimulating increased production of **1,25(OH)$_2$D.**
   c. In **bone,** PTH stimulates **bone resorption** (alters osteoclasts) **or bone formation** (alters osteoblasts); elevated PTH increases bone resorption.
   d. Combined effects cause
      1) **Serum:** Calcium increased, phosphate reduced
      2) **Urine:** Phosphate increased, calcium increased due to larger filtered load overriding increased tubular reabsorption
   e. **Increase in serum free calcium reduces secretion of PTH** through negative feedback, conversely decrease in serum free calcium stimulates secretion of PTH.

3. PTH quantified in plasma (EDTA preferred—stabilizes PTH) by measuring different forms of the hormone: **intact PTH, N-terminal PTH, mid-molecule PTH, and C-terminal PTH.**
   a. Electrochemiluminescence immunoassay (ECLIA)
   b. Measures **intact PTH** using a sandwich technique
   c. Reference range: 15–65 pg/mL
4. Measurement of PTH **during surgery for adenoma resection of the parathyroid glands** assists the surgeon in determining completeness of the resection **based on the rapid fall of PTH.** Need pre-incision baseline sample as surgery starts, second baseline sample following exposure of the gland, and post-excision sample drawn 10 minutes following gland removal. At 10 minutes post-excision, the PTH level should fall to 50% or less of the pre-incision value or the value at the time of gland resection. If the PTH remains increased and such a decrease does not occur or if the PTH rises again after what initially appeared to be a decrease, multigland disease or ectopic production needs to be investigated.
5. **Clinical significance**
   a. **Hyperparathyroidism**
      1) **Primary hyperparathyroidism** (results in increased blood calcium) may be caused by parathyroid adenoma (tumor), parathyroid carcinoma, or hyperplasia.
      2) **Secondary hyperparathyroidism** may be caused by vitamin D deficiency (presents with low blood calcium levels) or chronic renal failure.
   b. **Hypoparathyroidism** (results in decreased calcium and increased phosphate blood levels) may be caused by osteomalacia, autoimmune disease, inborn errors of metabolism, or unintentional removal during thyroid surgery.

K. Gastrointestinal Hormones: Overview and Clinical Significance

1. **Gastrin** is secreted by the stomach in response to the vagus and food entering the stomach. Maximum secretion occurs in the stomach at pH 5–7.
   a. **Function:** Gastrin stimulates secretion of gastric HCl and pancreatic enzymes.
   b. **Acidification** of the antrum of the stomach causes a **decrease in gastrin secretion.**
   c. **Zollinger-Ellison syndrome:** An **elevated gastrin** level accompanied by gastric hyperacidity; caused by gastrinomas, duodenal, or pancreatic endocrine tumors that secrete gastrin
2. **Serotonin** is synthesized from **tryptophan** and secreted by the enterochromaffin cells in the gastrointestinal tract.
   a. **Function:** Serotonin is a smooth muscle stimulant and vasoconstrictor that is transported by platelets.
   b. Liver **metabolizes serotonin to 5-hydroxyindole acetic acid** (5-HIAA).
      1) **Metastatic carcinoid tumors** occur in the appendix, ileum, or rectum.
      2) Produce excessive amount of **serotonin** and its metabolite **5-HIAA,** which is measured in urine.

L. Pancreas: Overview and Clinical Significance

1. **Pancreas** has both endocrine and exocrine functions
   a. **Endocrine function:** Islets of Langerhans secrete insulin, glucagon, gastrin, and somatostatin into the blood.
   b. **Exocrine function:** Digestive fluid containing bicarbonate and digestive enzymes is made in the acinar cells and secreted into the duodenum. Digestive enzymes include lipase, amylase, trypsin, chymotrypsin, elastase, collagenase, leucine aminopeptidase, and nucleases.
      1) **Secretion** of the digestive fluid is regulated by the vagus nerve and the endocrine hormones cholecystokinin and secretin.
2. **Insulin** is synthesized in the islets of Langerhans by the β-cells and secreted into the blood when the blood glucose level is elevated.
   a. **Insulin lowers blood glucose** by binding to cell membrane receptors, which increases membrane permeability in the liver, muscle, and adipose tissue. Insulin affects glucose metabolism by promoting glycogenesis and lipogenesis while inhibiting glycogenolysis.
   b. Insulin is **inhibited** by epinephrine and norepinephrine release and certain drugs (e.g., thiazide, dilantin, diazoxide).
   c. **Clinical significance**
      1) **Hyperinsulinemia:** May be caused by insulinomas (insulin-producing tumors of the β-cells of the pancreas), which result in hypoglycemia
      2) **Hypoinsulinemia:** Lack of insulin or ineffective insulin, which results in diabetes mellitus

3. **Glucagon** is synthesized in the islets of Langerhans by the α-cells and secreted into the blood when the blood glucose level is low. Glucagon increases blood glucose by promoting glycogenolysis in the liver and gluconeogenesis.
   a. The secretion of glucagon is promoted by exercise, stress, and amino acids.
   b. Secretion is **inhibited** by insulin.
   c. **Clinical significance**
      1) **Hyperglucagonemia** is associated with glucagon-secreting tumors of the pancreas. These tumors are malignant and have usually metastasized by the time they are diagnosed.

XI. THERAPEUTIC DRUG MONITORING

A. Therapeutic Drug Monitoring (TDM) entails the analysis, interpretation, and evaluation of drug concentration in serum, plasma, or whole blood samples.

1. **Purpose:** TDM is employed to establish maximum benefits with minimal toxic effects for drugs whose correlation with dosage, effect, or toxicity is not clear.
2. **Common routes of drug administration:** Oral, IV (intravenous), IM (intramuscular), and SC (subcutaneous)
3. **Therapeutic range:** Drug concentration that produces benefits

B. Drug Absorption and Distribution

1. Most drugs are **absorbed** from the **GI tract** in a consistent manner in healthy individuals.
2. **Liquids are absorbed more quickly** than tablets and capsules.
3. **First-pass metabolism:** All drugs absorbed from the GI tract must go through the liver before entering the general circulation.
4. **Most drugs circulate** in the blood **bound to plasma proteins.** A number of disorders may affect drug-protein binding, including kidney disease, hepatic disease, malnutrition, and inflammatory processes. In addition, drugs compete with other ingested drugs, as well as endogenous molecules such as the steroids and bilirubin, for protein binding sites.
   a. **Acidic drugs** primarily bind to **albumin.**
   b. **Basic drugs** primarily bind to **α_1-acid glycoprotein** (AAG).
   c. Some drugs **bind to both** albumin and AAG.
5. Only **free drugs** can **interact with target sites and produce a response.** Thus, the quantity of free drug correlates the best with monitoring therapeutic and toxic effects. Most TDM assays **quantify total drug** concentration rather than free drug.

6. **Measuring the free drug level** may be warranted for highly protein bound drugs or when clinical response is not consistent with the total drug level.
7. In general, drugs are **eliminated** from the circulation through **hepatic metabolic processes and renal filtration.** In the liver, drugs are chemically altered to metabolites, and they are conjugated to make them water soluble. Conjugated drugs can be eliminated through the urine or the bile.
8. Drugs are usually **administered in a scheduled manner** with multiple doses administered over a period of time. This manner of drug administration produces high (**peak** drug level) and low (**trough** drug level) variations in drug concentration. The aim is to keep the trough level from dropping below a concentration of **therapeutic benefit** and to keep the peak concentration from rising to the **toxic level.** Approximately **seven doses** of a drug are required to achieve a **steady state** where peak and trough levels can be assessed.

C. Sample Collection and Measurement

1. **Timing of blood sample collection is critical in TDM.**
2. When the **trough level** is required, the blood sample should be drawn right **before next dose** is administered.
3. Sample collection for **peak levels**
   a. Drawing blood sample **1 hour after oral administration** is the rule of thumb. However, collection time varies and is drug specific; variations in peak levels occur due to different absorption, metabolic, and excretion rates for individual drugs.
   b. Draw blood sample 0.5 hour after completion of IV administration.
4. Most drugs can be quantified using immunoassay techniques or chromatography (e.g., GC and HPLC).

D. Cardioactive Drugs

1. **Digoxin**
   a. **Function:** Cardiac glycoside used to treat **congestive heart failure**
   b. **Mechanism of action:** Digoxin inhibits membrane Na^+-K^+-ATPase, causing decrease in intracellular K^+ and increase in intracellular Ca^{2+} in cardiac myocytes; increased Ca^{2+} improves contraction of cardiac muscle. Electrolytes need to be monitored because digoxin function is enhanced by a low serum K^+ level.
   c. **Metabolism:** Digoxin levels need to be monitored to ensure blood concentrations are therapeutic because absorption of the drug is variable. Although the blood level **peaks in 2–3 hours** following oral ingestion, **tissue uptake** of digoxin is **slow,** making it necessary to monitor serum 8 hours after an oral dose, which correlates better with the tissue level.
   d. **Therapeutic range:** 0.8–2.0 ng/mL

2. **Lidocaine**
   a. **Function: Antiarrhythmic** drug used to treat ventricular arrhythmia and prevent ventricular fibrillation
   b. **Metabolism:** Lidocaine is usually given by **continuous IV administration** after a loading bolus, and it is primarily metabolized by the liver to the metabolic by-product monoethylglycinexylidide (MEGX). Although **MEGX** does not contribute to therapeutic effect, it does **enhance toxicity.** Hence the **need to measure both lidocaine and MEGX;** some immunoassays are able to quantify both. Oral administration is contraindicated, because lidocaine would be removed from the circulation during the first pass through the liver.
   c. **Therapeutic range:** 1.5–4.0 μg/mL
   d. **Toxicity:** Individuals with blood levels of 4–8 μg/mL exhibit CNS depression and >8 μg/mL exhibit seizures and severe hypotension.
3. **Quinidine**
   a. **Function: Antiarrhythmic** drug used to treat cardiac arrhythmia
   b. **Metabolism:** Quinidine may be administered orally as the sulfate or gluconate form, and it is primarily metabolized by the liver. **Quinidine sulfate** is absorbed more quickly than the gluconate form, with **peak** plasma levels occurring **2 hours** after oral ingestion. In contrast, **peak** plasma levels of **quinidine gluconate** occur in **4–5 hours.**
   c. Usually, the **trough level** is monitored to ensure achievement of therapeutic levels. In the case of quinidine gluconate administration, sample collection is performed 1 hour following the last ingested dose for trough determination because of its slow absorption rate.
   d. **Therapeutic range:** 2–5 μg/mL
4. **Procainamide**
   a. **Function: Antiarrhythmic** drug used to treat cardiac arrhythmia
   b. **Metabolism:** Procainamide is administered orally, with elimination dependent on it being metabolized by the liver and filtered by the kidney. Procainamide is **metabolized to *N*-acetylprocainamide (NAPA),** which exhibits a similar physiological effect as the parent drug. Thus, it is necessary to **quantify both** procainamide and NAPA when assessing serum concentration. **Peak plasma levels** occur approximately 1 hour after ingestion.
   c. **Therapeutic range:** 4–8 μg/mL

E. Antibiotic Drugs

1. **Aminoglycosides**
   a. **Function:** Used to **treat infections** caused by **gram-negative** bacteria; include gentamicin, tobramycin, kanamycin, and amikacin
   b. **Metabolism:** The aminoglycosides are administered IV or IM because gastrointestinal absorption is poor. Elimination is via kidney filtration.
   c. Associated with nephrotoxicity and ototoxicity

2. **Vancomycin**
   a. **Function:** Used to **treat infections** caused by **gram-positive** bacteria
   b. **Metabolism:** Administered by IV because of poor gastrointestinal absorption
   c. May be associated with nephrotoxicity, ototoxicity, and "red-man syndrome" (erythemic flushing of extremities)

F. Antiepileptic Drugs

1. **Phenobarbital**
   a. **Function:** Slow-acting barbiturate used to **control seizures**
   b. **Metabolism:** Phenobarbital is administered orally with a **peak** plasma level occurring at **10 hours** following ingestion. It is characterized by slow absorption and a long half-life. Elimination is dependent on it being metabolized by the liver and filtered by the kidney.
   c. **Primidone** is the **inactive form** of phenobarbital, and this proform is administered when rapid absorption is indicated. **Primidone** is quickly **converted** to phenobarbital. When primidone is administered, both compounds need to be quantified.
   d. **Toxicity** effects are drowsiness, depression, fatigue, and altered mental ability.
   e. **Therapeutic range:** 15–40 μg/mL
2. **Phenytoin** (diphenylhydantoin)
   a. **Function:** Used to **control seizures** and to keep the brain from swelling and injuring tissue during brain traumas
   b. **Metabolism:** Phenytoin, administered orally with slow GI absorption, has low solubility in aqueous solutions; thus it is 90–95% protein bound in the circulation. The small free component of this drug is physiologically active. Drug elimination is controlled by liver metabolism. For **trough** levels, the sample is **drawn before the next dose** is ingested. For **peak** levels when toxicity is a concern, the sample is **drawn 4–5 hours after the last dose.**
   c. Toxicity characterized by seizures
   d. **Therapeutic range:** Total serum level 10–20 μg/mL; free serum level 1–2 μg/mL
   e. **Fosphenytoin: IM** injectable proform of the drug
3. **Valproic acid**
   a. **Function:** Used to **control seizures**
   b. **Metabolism:** Administered orally, 93% protein bound in the circulation, and metabolized by the liver for elimination
   c. **Therapeutic range:** 50–100 μg/mL
4. **Carbamazepine**
   a. **Function:** Used to **control seizures**

b. **Metabolism:** Administered orally, 70–80% protein bound in the circulation, and metabolized by the liver for elimination
c. **Therapeutic range:** 4–12 μg/mL

G. Antipsychotic Drugs

1. **Lithium**
   a. **Function:** Used to treat **manic depression**
   b. **Metabolism:** Administered orally as lithium carbonate, does not bind to plasma proteins in the circulation, **peak** plasma levels occur **2–4 hours** after oral ingestion, and filtered by the kidney for elimination
   c. **Therapeutic range:** 1.0–1.2 mmol/L
2. **Tricyclic antidepressants** (TCAs)
   a. **Function:** TCAs include amitriptyline, imipramine, and doxepin, which may be used in cases of depression, insomnia, extreme apathy, and loss of libido.
   b. **Metabolism:** TCAs are administered orally, but GI absorption is slow. This results in **peak** concentrations occurring **2–12 hours after ingestion.** TCAs are metabolized by the liver for elimination. Amitriptyline and imipramine are metabolized to the **active metabolites nortriptyline and desipramine,** respectively.
   c. **Therapeutic range:** Amitriptyline 120–150 ng/mL; imipramine 150–300 ng/mL; nortriptyline 50–150 ng/mL; desipramine 150–300 ng/mL

H. Bronchodilator Drugs

1. **Theophylline**
   a. **Function:** Used to treat **asthma** and other chronic obstructive pulmonary disorders (COPD)
   b. **Metabolism:** Administered orally, with elimination dependent on it being metabolized by the liver and filtered by the kidney
   c. **Therapeutic range:** 10–20 μg/mL

I. Immunosuppressive Drugs

1. **Cyclosporine**
   a. **Function:** Used to **suppress transplant rejections** and **graft-versus-host disease**
   b. **Metabolism:** Administered orally with **peak** levels reached in **4–6 hours;** elimination dependent on it being metabolized by the liver
   c. **Therapeutic range:** Specimen of choice is **whole blood.** Therapeutic ranges vary with organ transplanted; liver, pancreas, and heart require 200–350 ng/mL, and renal transplants require 100–300 ng/mL.
2. **Tacrolimus** (Prograf)
   a. **Function:** Used to **suppress transplant rejections** and **graft-versus-host disease** (potency far exceeds cyclosporine by a factor of 100)

b. **Metabolism:** Administered orally, with elimination dependent on it being metabolized by the liver
c. **Therapeutic range:** 10–15 ng/mL, with therapeutic ranges varying with organ transplanted; specimen of choice, **whole blood**

3. **Sirolimus** (Rapamune)
   a. **Function:** Used to **suppress transplant rejections** and **graft-versus-host disease**
   b. **Metabolism:** Administered orally with peak levels reached in about 2 hours; elimination dependent on it being metabolized by the liver
   c. **Therapeutic range:** 4–12 ng/mL with therapeutic ranges varying with organ transplanted; specimen of choice **whole blood**

J. Antineoplastic Drugs

1. **Methotrexate**
   a. **Function:** Methotrexate is used to **destroy neoplastic cells.** Although methotrexate inhibits the synthesis of DNA in all cells, its action is based on the principle that neoplastic cells contain a greater amount of DNA because of their rapid rate of division as compared to normal cells. Thus, neoplastic cells are more susceptible to the loss of DNA.
   b. **Metabolism:** May be administered IV; filtered by the kidney for elimination
   c. **Leucovorin rescue** refers to the administration of leucovorin to offset the effect of methotrexate in an attempt to prevent cytotoxicity of normal cells. Leucovorin dosage is based on the amount of methotrexate in the circulation.
   d. **Therapeutic monitoring:** High-dose therapy generally exceeds 50 mg/m^2, and serum levels vary with the time interval following dosage. Serum levels of methotrexate are monitored at 24, 48, and 72 hours after drug administration.

XII. TOXICOLOGY

A. Elements of Toxicology

1. **Toxicology:** The **study** of poisonous substances
2. **Exposure to toxins:** May be due to suicide attempt, accidental exposure, or occupational exposure
3. **Routes of exposure:** Ingestion, inhalation, and transdermal absorption
4. **Toxic response:** The amount of damage done to an organism when the substance is administered at less than the lethal dose
5. **Acute toxicity:** A one-time exposure of short duration to an agent that immediately causes a toxic response
6. **Chronic toxicity:** Multiple exposures for extended time periods to an agent at a dosage that will not cause an acute response

B. Analysis of Toxic Agents

1. **Screening test:** Performed first and usually of a qualitative nature; may lack specificity
2. **Confirmatory test:** Usually quantitative with good specificity and sensitivity (e.g., gas chromatography and immunoassays)

C. Analysis of Specific Substances

1. **Alcohols:** Volatile organic substances
   a. Types of alcohols
      1) **Ethanol:** Chronic exposure is associated with toxic hepatitis and cirrhosis.
      2) **Methanol:** Ingestion is related to severe acidosis, blindness, and even death due to methanol conversion to formaldehyde, which is metabolized to formic acid.
      3) **Isopropanol:** Ingestion produces severe, acute symptoms, similar to ethanol intoxication, that persist for a long period of time because isopropanol is metabolized to acetone, which has a long half-life.
      4) **Ethylene glycol** (found in antifreeze): Ingestion produces severe metabolic acidosis and renal tubular damage.
   b. **Analysis for ethanol:** Enzymatic and gas-liquid chromatography
      1) $\text{Ethanol} + \text{NAD}^+ \xrightarrow{\text{ADH}} \text{acetaldehyde} + \text{NADH}$
      2) Gas-liquid chromatography (GLC) is the reference method. GLC can differentiate among the various types of alcohols and quantify them.
2. **Carbon monoxide**
   a. Toxic because it **binds very tightly to hemoglobin** and does not allow oxygen to attach to the hemoglobin; forms carboxyhemoglobin
   b. Produces **hypoxia** in brain and heart
   c. Whole blood is required for analysis.
   d. **Analysis:** Gas-liquid chromatography is the reference method.
3. **Cyanide**
   a. **Supertoxic** substance with exposure occurring through various routes, including oral ingestion, inhalation, or transdermal absorption
   b. Used in insecticide and rodenticide products
   c. Cyanide **binds** to heme iron and mitochondrial cytochrome oxidase.
   d. **Analysis:** Ion-selective electrode
4. **Metals**
   a. **Arsenic**
      1) **Binds to thiol groups** in proteins; ionized arsenic excreted in urine
      2) **Specimens:** Blood and urine used to assess short-term exposure; hair and fingernails used to assess long-term exposure
      3) **Analysis:** Atomic absorption spectrophotometry
   b. **Lead**

1) **Lead binds to proteins** and it inhibits many enzymes; it also inhibits heme synthesis. Toxicity may occur when lead is ingested, inhaled, or contacted dermally.
2) Lead **interferes** in heme biosynthesis at several stages, the last of these being the incorporation of **iron** into the tetrapyrrole ring. This alteration results in the formation and accumulation of **zinc protoporphyrin (ZPP),** with zinc replacing the iron in the tetrapyrrole ring.
3) **Lead poisoning** in children is generally associated with the ingestion of lead-laden paint chips. Laboratory results indicate:
   a) **Basophilic stippling** of RBCs
   b) Increased urinary excretion of **aminolevulinic acid** and **coproporphyrins**
4) **Acceptable blood lead level:** <10 μg/dL in young children
5) **Lead analysis: Whole blood** specimen required; methods include atomic absorption spectrophotometry and anodic stripping voltammetry

c. **Mercury**
   1) **Binds to proteins** and inhibits many enzymes
   2) **Analysis:** Atomic absorption spectrophotometry and anodic stripping voltammetry

5. **Pesticides**
   a. Include insecticides and herbicides that may contaminate food or be inhaled, absorbed through the skin, and ingested via hand-to-mouth contact
   b. Organophosphate and carbamate insecticides **inhibit acetylcholinesterase.**
   c. **Analysis:** Assess enzyme activity of erythrocyte acetylcholinesterase or serum pseudocholinesterase.
6. **Therapeutic drugs commonly abused**
   a. **Salicylate** (aspirin)
      1) **Function:** Used as an analgesic, antipyretic, and anti-inflammatory
      2) **Metabolism:** Administered orally
      3) **Toxic effects at high dosages:** Causes mixed acid-base imbalance seen as metabolic acidosis and respiratory alkalosis (respiratory center stimulant), ketone body formation, excess formation of lactate
      4) **Analysis:** Ferric nitrate method with colored product read spectrophotometrically; gas or liquid chromatography
   b. **Acetaminophen** (Tylenol)
      1) **Function:** Used as an analgesic
      2) **Metabolism:** Administered orally, with elimination dependent on it being metabolized by the liver
      3) **Toxic effect at high dosages:** Liver toxicity
      4) **Analysis:** Immunoassays and high-performance liquid chromatography

7. **Drugs of abuse**
   a. **Amphetamine and methamphetamine**
      1) **Function:** Used to treat narcolepsy and disorders that affect ability to focus; stimulants, provide sense of mental and physical well-being
      2) **Analysis:** Immunoassays and gas or liquid chromatography
   b. **Anabolic steroids**
      1) **Function:** Used to increase muscle mass and athletic performance
      2) **Analysis:** Gas or liquid chromatography
   c. **Cannabinoids**
      1) **Function:** Hallucinogenic, provide a feeling of mental well-being and euphoria, impair mental function and short-term memory
      2) **Marijuana: Tetrahydrocannabinol (THC)** primary cannabinoid component; THC half-life in blood is one day following single use and 3–5 days following chronic use
      3) **Metabolism:** THC distributes in lipophilic tissue such as the brain and adipose tissue. Elimination is dependent on THC being metabolized by the liver to 11-nor-Δ-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) with this metabolic product filtered by the kidney. **THC-COOH** (major urinary metabolite) is detectable in urine for 3–5 days following single use and for as long as 4 weeks following chronic use.
      4) **Analysis:** Immunoassays and gas chromatography/mass spectrometry
   d. **Cocaine**
      1) **Function:** Used as a local anesthetic and at higher levels functions as a CNS stimulant
      2) **Metabolism:** Half-life 0.5–1 hour, with elimination dependent on liver metabolism; **benzoylecgonine** (half-life 4–7 hours) **primary metabolite** and filtered by the kidney
      3) **Benzoylecgonine** is detectable in urine for 3 days following single use and for as long as 20 days following chronic use.
      4) **Analysis:** Immunoassays and gas chromatography/mass spectrometry
   e. **Opiates**
      1) Types of opiates
         a) **Naturally occurring:** Opium, morphine, codeine
         b) **Chemically modified:** Heroin, dilaudid, oxycodone
         c) **Synthetic:** Demerol, methadone, darvon, talwin, fentanyl
      2) **Function:** Narcotics used for their analgesic, sedative, and anesthetic properties
      3) **Metabolism:** Respiratory center depressant causing respiratory acidosis
      4) **Analysis:** Immunoassays and gas chromatography/mass spectrometry
   f. **Phencyclidine** (PCP)
      1) **Function:** Produces stimulant, depressant, anesthetic, and hallucinogenic effects

2) **Metabolism:** Distributes in lipophilic tissue such as the brain and adipose tissue; elimination dependent on it being metabolized by the liver, with 10–15% of the parent compound filtered by the kidney; detectable in urine for as long as 7–30 days following chronic use
3) **Analysis:** Immunoassays and gas chromatography/mass spectrometry

g. **Tranquilizers**
1) Types of tranquilizers
a) **Barbiturates:** Phenobarbital (long acting), amobarbital (intermediate acting), and secobarbital and pentobarbital (short acting)
b) **Benzodiazepines:** Diazepam (valium), chlordiazepoxide (librium), lorazepam (ativan)
2) **Function:** Sedative hypnotics that produce depression of the CNS
3) **Metabolism:** Respiratory center depressants causing respiratory acidosis
4) **Analysis:** Immunoassays and gas-liquid chromatography

XIII. VITAMINS

A. Solubility

1. **Fat-soluble** vitamins include A, D, E, and K.
2. **Water-soluble** vitamins include C, ascorbic acid; B_1, thiamin; B_2, riboflavin; B_6, pyridoxine; B_{12}, cobalamin; niacin, nicotinic acid; pantothenic acid; biotin; folate, folic acid.

B. Metabolism

1. Fat-soluble vitamins **stored in liver or adipose tissue;** may accumulate to toxic levels
2. Water-soluble vitamins **easily excreted in urine;** generally do not accumulate to toxic levels

C. Clinical Significance of Vitamins

1. **Vitamin A deficiency:** Drying, degeneration, and increased risk of infection in conjunctiva, cornea, skin, and mucous membranes; night blindness
2. **Vitamin D deficiency:** Rickets, osteomalacia, osteoporosis
3. **Vitamin E deficiency:** Hemolytic disease of premature neonates
4. **Vitamin K deficiency:** Hemorrhage
5. **Vitamin C deficiency:** Scurvy, necrosis of gums, emotional disturbances
6. **Vitamin B_1 deficiency:** Beriberi
7. **Vitamin B_2 deficiency:** Cheilosis, angular stomatitis, glossitis, seborrheic dermatitis, ocular disturbances
8. **Vitamin B_6 deficiency:** Eczema, seborrheic dermatitis, cheilosis, glossitis, angular stomatitis, mental depression, anemia

9. **Vitamin B_{12} deficiency:** Hematologic effects, including macrocytic anemia, and neurologic effects, including peripheral nerve degeneration
10. **Niacin deficiency:** Pellagra
11. **Pantothenic acid deficiency:** Metabolism affected; causes nausea, vomiting, muscular weakness, malaise
12. **Biotin deficiency:** Cutaneous, ophthalmic, and neurologic symptoms
13. **Folate deficiency:** Megaloblastic anemia, anorexia, glossitis, nausea, hepatosplenomegaly, hyperpigmentation of skin

D. Methods for Quantification

1. A number of methods exist for quantifying vitamins, including fluorometric assays, HPLC, liquid chromatography–tandem mass spectrometry, competitive protein-binding assays, immunoassays, bioassays, microbiological assays, enzyme activation tests, spectrophotometric, electrochemical, and RIA.

review questions

INSTRUCTIONS Each of the questions or incomplete statements that follow is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

Instrumentation and Analytical Principles

1. Which of the following lamps provides a continuous spectrum of radiant energy in the visible, near IR, and near UV regions of the spectrum?
   A. Tungsten-filament
   B. Hydrogen
   C. Deuterium
   D. Mercury vapor
2. Which of the following isolates light within a narrow region of the spectrum?
   A. Photomultiplier tube
   B. Monochromator
   C. Photovoltaic cell
   D. Detector
3. Which of the following is *not* descriptive of a photomultiplier tube?
   A. Emits electrons proportionally to initial light absorbed
   B. Must be shielded from stray light
   C. Cannot be used with a chopper
   D. Amplifies the initial signal received
4. Which of the following is *false* about a photomultiplier tube?
   A. Converts radiant energy (light) to electrical energy (current)
   B. Amplifies the current significantly
   C. Has a very rapid response time
   D. Is composed of an iron plate and a layer of selenium
5. Which type of photodetector employs a linear arrangement that allows it to respond to a specific wavelength resulting in complete UV/visible spectrum analysis?
   A. Photomultiplier tube
   B. Phototube
   C. Barrier layer cell
   D. Photodiode array
6. When performing spectrophotometer quality assurance checks, what is the holmium oxide glass filter used to assess?
   A. Linearity
   B. Stray light
   C. Absorbance accuracy
   D. Wavelength accuracy

7. In spectrophotometric analysis, what is the purpose of the reagent blank?
   A. Correct for interfering chromogens
   B. Correct for lipemia
   C. Correct for protein
   D. Correct for color contribution of the reagents

8. In regard to bichromatic analysis, which of the following is *false?*
   A. Absorbance is measured at the spectral absorbance peak for a blank and the sample using the same wavelength.
   B. Eliminates background interferences
   C. Sample concentration determined from difference in two measured absorbances
   D. Functions as a reference blank for each sample

9. The bandpass of a spectrophotometer is 10 nm. If an instrument is set at 540 nm, the wavelengths that are permitted to impinge on the sample will be within what wavelength range?
   A. 530–540 nm
   B. 530–550 nm
   C. 535–545 nm
   D. 540–550 nm

10. Which of the following formulas is an expression of the Beer-Lambert law that is routinely applied to spectrophotometric analysis?
    A. $A_u \times \frac{C_s}{A_s} = C_u$
    B. $C_u \times \frac{C_s}{A_s} = A_u$
    C. $A_s \times \frac{C_s}{C_u} = A_u$
    D. $A = 2 - \log \%T$

11. In spectrophotometry, which of the following is a mathematical expression of the relationship between absorbance and transmittance?
    A. $A = abc$
    B. $\frac{A_u}{C_u} = \frac{A_s}{C_s}$
    C. $A = 2 - \log \%T$
    D. $A = \log \%T$

12. Which of the following is *not* a problem inherent in turbidimetry?
    A. Variation in particle size of samples
    B. Variation in particle size of standards
    C. Rate of aggregation or settling of particles
    D. Need to maintain a constant and specific temperature

13. Which of the following may be associated with reflectance spectrophotometry as it relates to the dry reagent slide technique?
    A. Light projected to the slide at 180-degree angle
    B. Dye concentration directly proportional to reflectance
    C. Unabsorbed, reflected light detected by photodetector
    D. Reflectance values are linearly proportional to transmission values

14. Fluorometers are designed so that the path of the exciting light is at a right angle to the path of the emitted light. What is the purpose of this design?
    A. Prevent loss of emitted light
    B. Prevent loss of the excitation light
    C. Focus emitted and excitation light upon the detector
    D. Prevent excitation light from reaching the detector

15. Which of the following represents a primary advantage of performing fluorometric over absorption spectroscopic methods of analysis?
    A. Increased specificity and increased sensitivity
    B. Increased specificity and decreased sensitivity
    C. Purity of reagents used not as critical
    D. Ease of performing assays

16. Which of the following may be associated with fluorescence polarization?
    A. Plane-polarized light is used for sample excitation.
    B. Small molecular complexes show a greater amount of polarization.
    C. It is a heterogeneous technique employed in fluorophore-ligand immunoassays.
    D. Polarized light detected is directly proportional to concentration of ligand in sample.

17. Which of the following may be associated with bioluminescence?
    A. Light emission produced due to enzymatic oxidation of a substrate
    B. Less sensitive than direct fluorescent assays
    C. Electron excitation caused by radiant energy
    D. Employs a radioactive label

18. Nephelometry is based on the measurement of light that is
    A. Absorbed by particles in suspension
    B. Scattered by particles in suspension
    C. Produced by fluorescence
    D. Produced by excitation of ground-state atoms

19. Which of the following instruments is used in the clinical laboratory or in reference laboratories to detect beta and gamma emissions?
    A. Fluorometer
    B. Nephelometer
    C. Scintillation counter
    D. Spectrophotometer

20. Which of the following best describes chemiluminescence?
    A. Electron excitation caused by radiant energy
    B. Enzymatic oxidation of a substrate produces light emission
    C. Chemical energy excites electrons that emit light upon return to ground state
    D. Employs a fluorescent label that produces light

21. In assaying an analyte with a single-beam atomic absorption spectrophotometer, what is the instrument actually measuring?
    A. Intensity of light emitted by the analyte on its return to the ground state
    B. Intensity of light that the analyte absorbs from the hollow-cathode lamp
    C. Intensity of light that the analyte absorbs from the flame
    D. Intensity of the beam from the hollow-cathode lamp after it has passed through the analyte-containing flame

22. What is the function of the flame in atomic absorption spectroscopy?
    A. Absorb the energy emitted from the metal analyte in returning to ground state
    B. Supply the thermal energy needed to excite the metal analyte
    C. Bring the metal analyte to its ground state
    D. Supply the light that is absorbed by the metal analyte

23. Most atomic absorption spectrophotometers incorporate a beam chopper and a tuned amplifier. The purpose of these components is to avoid errors that would be caused by
    A. Variations in flame temperature
    B. Deterioration of the hollow-cathode lamp
    C. Stray light from the hollow-cathode lamp
    D. Measurement of light emitted by the analyte

24. In potentiometry, which of the following is considered the standard electrode?
    A. Hydrogen electrode
    B. Calcium electrode
    C. Potassium electrode
    D. Copper electrode

25. In an electrolytic cell, which of the following is the half-cell where reduction takes place?
    A. Anode
    B. Cathode
    C. Combination electrode
    D. Electrode response

26. Mercury covered by a layer of mercurous chloride in contact with saturated potassium chloride solution is a description of which of the following types of electrodes?
    A. Sodium
    B. Calomel
    C. Calcium
    D. Silver/silver chloride

27. When a pH-sensitive glass electrode is not actively in use, in what type of solution should it be kept?
    A. Tap water
    B. Physiologic saline solution
    C. The medium recommended by the manufacturer
    D. A buffer solution of alkaline pH

28. When measuring K^+ with an ion-selective electrode by means of a liquid ion-exchange membrane, what antibiotic will be incorporated into the membrane?
    A. Monactin
    B. Nonactin
    C. Streptomycin
    D. Valinomycin

29. Which of the following is *false* about ion-selective electrode analysis of sodium?
    A. Uses a glass membrane
    B. Errors occur from protein buildup on the membrane.
    C. Membrane coated with valinomycin
    D. Principle based on potentiometry

30. What are the principles of operation for a chloride analyzer that generates silver ions as part of its reaction mechanism?
    A. Potentiometry and amperometry
    B. Amperometry and polarography
    C. Coulometry and potentiometry
    D. Amperometry and coulometry

31. When quantifying glucose using an amperometric glucose electrode system, which of the following is *not* a component of the system?
    A. Product oxidation produces a current
    B. Hydrogen peroxide formed
    C. Hexokinase reacts with glucose
    D. Platinum electrode

32. To calibrate the pH electrode in a pH/blood gas analyzer, it is necessary that
    A. The barometric pressure be known and used for adjustments
    B. Calibrating gases of known high and low concentrations be used
    C. The calibration be performed at room temperature
    D. Two buffer solutions of known pH be used

33. The measurement of CO_2 in blood by means of a PCO_2 electrode is dependent on the
    A. Passage of H^+ ions through the membrane that separates the sample and the electrode
    B. Change in pH because of increased carbonic acid in the electrolyte surrounding the electrodes
    C. Movement of bicarbonate across the membrane that separates the sample and the electrode
    D. Linear relationship between PCO_2 in the sample and measured pH

34. The measurement of oxygen in blood by means of a PO_2 electrode involves which of the following?
    A. Wheatstone bridge arrangement of resistive elements sensitive to oxygen concentration
    B. Direct relationship between amount of oxygen in the sample and amount of current flowing in the measuring system
    C. Change in current resulting from an increase of free silver ions in solution
    D. Glass electrode sensitive to H^+ ions

35. Which of the following blood gas parameters are measured directly by the blood gas analyzer electrochemically as opposed to being calculated by the instrument?
    A. pH, HCO_3^-, total CO_2
    B. PCO_2, HCO_3^-, PO_2
    C. pH, PCO_2, PO_2
    D. PO_2, HCO_3^-, total CO_2

36. Which of the following statements is *false* about anodic stripping voltammetry (ASV)?
    A. Based on potentiometry
    B. Occurs in an electrochemical cell
    C. Involves preconcentration of the analyte by electroplating
    D. Used to measure lead

37. Which of the following methods allows for the separation of charged particles based on their rates of migration in an electric field?
    A. Rheophoresis
    B. Electrophoresis
    C. Electroendosmosis
    D. Ion exchange

38. Which of the following techniques is based on electro-osmotic flow?
    A. Capillary electrophoresis
    B. Zone electrophoresis
    C. Iontophoresis
    D. Isoelectric focusing

39. Which of the following is *not* a type of support media used for serum protein electrophoresis?
    A. Agarose gel
    B. Cellulose acetate
    C. Acrylamide
    D. Celite

40. In serum protein electrophoresis, when a buffer solution of pH 8.6 is used, which of the following characterizes the proteins?
    A. Exhibit net negative charge
    B. Exhibit net positive charge
    C. Exhibit charge neutrality
    D. Migrate toward the cathode

41. Which of the following characteristics will a protein have at its isoelectric point?
    A. Net negative charge
    B. Net positive charge
    C. Net zero charge
    D. Mobility

42. What dye may be used for staining protein bands following electrophoresis?
    A. Fat red 7B
    B. Sudan black B
    C. Ponceau S
    D. Oil red O

43. When electrophoresis is performed, holes appear in the staining pattern, giving the stained protein band a doughnut-like appearance. What is the probable cause of this problem?
    A. Protein denatured and will not stain properly
    B. Ionic strength of the buffer was too high
    C. Protein reached its isoelectric point and precipitated out
    D. Protein concentration was too high

44. What is the purpose of using ampholytes in isoelectric focusing?
    A. Maintain the polyacrylamide gel in a solid state
    B. Maintain the protein sample in a charged state
    C. Maintain the pH of the buffer solution
    D. Establish a pH gradient in the gel

45. Which of the following is *not* associated with silver stains?
    A. Reactive to nanogram concentrations of proteins
    B. Polypeptides stain a variety of colors
    C. Not as sensitive as Coomassie brilliant blue
    D. Preconcentration of CSF not necessary

46. Which of the following is *not* associated with isoelectric focusing?
    A. Continuous pH gradient
    B. Migration of proteins with net charge of zero
    C. Separation dependent on isoelectric point
    D. Zone electrophoresis

47. Which of the following is an electrophoretic technique employing a pH gradient that separates molecules with similar isoelectric points?
    A. Zone electrophoresis
    B. High-resolution electrophoresis
    C. Isoelectric focusing
    D. Immunoelectrophoresis

48. Given the following information on a particular compound that has been visualized by means of thin-layer chromatography, calculate the R_f of the compound.
    Distance from origin to spot center = 48 mm
    Distance from spot center to solvent front = 93 mm
    Distance from origin to solvent front = 141 mm
    A. 0.29
    B. 0.34
    C. 0.52
    D. 0.66

49. To achieve the best levels of sensitivity and specificity, to what type of detector system could a gas chromatograph be coupled?
    A. UV spectrophotometer
    B. Bichromatic spectrophotometer
    C. Mass spectrometer
    D. Fluorescence detector

50. Which of the following instruments has a sample-introduction system, solvent-delivery system, column, and detector as components?
    A. Atomic absorption spectrometer
    B. Mass spectrometer
    C. High-performance liquid chromatograph
    D. Nephelometer

51. Which type of elution technique may be used in high-performance liquid chromatography?
    A. Amphoteric
    B. Isoelectric
    C. Gradient
    D. Ion exchange

52. Which of the following statements best describes discrete analysis?
    A. Each sample-reagent mixture is handled separately in its own reaction vessel.
    B. Samples are analyzed in a flowing stream of reagent.
    C. Analyzer must be dedicated to measurement of only one analyte.
    D. It does not have random access capability.

53. Which of the following chromatography systems may be described as having a stationary phase that is liquid absorbed on particles packed in a column and a liquid moving phase that is pumped through a column?
    A. Thin-layer
    B. High-performance liquid
    C. Ion-exchange
    D. Gas-liquid

54. Which of the following chromatography systems is characterized by a stationary phase of silica gel on a piece of glass and a moving phase of liquid?
    A. Thin-layer
    B. Ion-exchange
    C. Gas-liquid
    D. Partition

55. Which of the following does *not* apply to gas-liquid chromatography?
    A. Separation depends on volatility of the sample.
    B. Separation depends on the sample's solubility in the liquid layer of the stationary phase.
    C. Stationary phase is a liquid layer adsorbed on the column packing.
    D. Mobile phase is a liquid pumped through the column.

56. Ion-exchange chromatography separates solutes in a sample based on the
    A. Solubility of the solutes
    B. Sign and magnitude of the ionic charge
    C. Adsorption ability of the solutes
    D. Molecular size

57. Which parameter is used in mass spectrometry to identify a compound?
    A. Ion mass-to-charge ratio
    B. Molecular size
    C. Absorption spectrum
    D. Retention time

58. Which chromatography system is commonly used in conjunction with mass spectrometry?
    A. High-performance liquid
    B. Ion-exchange
    C. Partition
    D. Gas-liquid

59. Which of the following may be a sampling source of error for an automated instrument?
    A. Short sample
    B. Air bubble in bottom of sample cup
    C. Fibrin clot in sample probe
    D. All the above

60. Checking instrument calibration, temperature accuracy, and electronic parameters are part of
    A. Preventive maintenance
    B. Quality control
    C. Function verification
    D. Precision verification

61. For which of the following laboratory instruments should preventive maintenance procedures be performed and recorded?
    A. Analytical balance
    B. Centrifuge
    C. Chemistry analyzer
    D. All the above

62. Which of the following is *not* the reason that preventive maintenance schedules are required?
    A. Keep instrument components clean
    B. Replace worn parts
    C. Extend the life of the equipment
    D. Keep personnel busy when the laboratory work is slow

63. Which globin chains compose hemoglobin A_1?
    A. Two alpha chains and two beta chains
    B. Two alpha chains and two delta chains
    C. Two alpha chains and two gamma chains
    D. Two beta chains and two delta chains

64. Which hemoglobin may be differentiated from other hemoglobins on the basis of its resistance to denature in alkaline solution?
    A. A_1
    B. A_2
    C. C
    D. F

65. Hemoglobin S is an abnormal hemoglobin that is characterized by a substitution of which amino acid?
    A. Valine for glutamic acid in position 6 on the beta chain
    B. Valine for glutamic acid in position 6 on the alpha chain
    C. Lysine for glutamic acid in position 6 on the beta chain
    D. Lysine for glutamic acid in position 6 on the alpha chain

66. When performing electrophoresis at pH 8.6, which hemoglobin molecule migrates the fastest on cellulose acetate toward the anode?
    A. A_1
    B. A_2
    C. F
    D. S

67. Because of similar electrophoretic mobilities, several hemoglobins cannot be differentiated on cellulose acetate medium. Electrophoresis of hemoglobins at pH 6.2 on agar gel may be useful in differentiating which hemoglobins?
    A. A_1 from A_2
    B. A_1 from D
    C. A_1 from E
    D. C from A_2

68. In addition to performing hemoglobin electrophoresis, a solubility test may be performed to detect the presence of what hemoglobin?
    A. A_1
    B. C
    C. F
    D. S

69. Which of the following is *not* quantified using an immunoassay method?
    A. Vitamins
    B. Hormones
    C. Electrolytes
    D. Drugs

70. Which of the following is a homogeneous immunoassay where separation of the bound from the free labeled species is *not* required?
    A. Radioimmunoassay
    B. Enzyme-linked immunosorbent assay
    C. Immunoradiometric assay
    D. Enzyme-multiplied immunoassay technique

71. The substance to be measured reacts with a specific macromolecule of limited binding capacity. Which of the following assays does *not* employ this principle?
    A. Chemiluminescence immunoassay
    B. Enzyme-multiplied immunoassay technique
    C. Fluorescent polarization immunoassay
    D. High-performance liquid chromatography

72. Which of the following is *not* associated with the enzyme-multiplied immunoassay technique (EMIT)?
    A. Is a homogeneous enzyme immunoassay
    B. Determines antigen concentration
    C. Employs a labeled reactant
    D. Enzyme reacts with drug in serum sample

73. When using EMIT, the enzyme is coupled to
    A. Antibody
    B. Antigen
    C. Substrate
    D. Coenzyme

74. The enzyme activity measured in the EMIT is the result of the reaction between the substrate and coenzyme with
    A. Free antibody
    B. Free unlabeled antigen
    C. Free labeled antigen
    D. Labeled antigen-antibody complexes

75. Singlet oxygen reacting with a precursor chemiluminescent compound to form a decay product whose light energizes a fluorophore best describes
    A. Fluorescent polarization immunoassay
    B. Enzyme-multiplied immunoassay technique
    C. Electrochemiluminescence immunoassay
    D. Luminescent oxygen channeling immunoassay

76. Which of the following stimulates the production of singlet oxygen at the surface of the sensitizer particle in a luminescent oxygen channeling immunoassay?
    A. Radiant energy
    B. Heat energy
    C. Enzymatic reaction
    D. Fluorescent irradiation

Proteins and Tumor Markers

77. Proteins, carbohydrates, and lipids are the three major biochemical compounds of human metabolism. What is the element that distinguishes proteins from carbohydrate and lipid compounds?
    A. Carbon
    B. Hydrogen
    C. Oxygen
    D. Nitrogen

78. Proteins may become denatured when subjected to mechanical agitation, heat, or extreme chemical treatment. How are proteins affected by denaturation?
    A. Alteration in primary structure
    B. Alteration in secondary structure
    C. Alteration in tertiary structure
    D. Increase in solubility

79. What is the basis for the Kjeldahl technique for the determination of serum total protein?
    A. Quantification of peptide bonds
    B. Determination of the refractive index of proteins
    C. Ultraviolet light absorption by aromatic rings at 280 nm
    D. Quantification of the nitrogen content of protein

80. When quantifying serum total proteins, upon what is the intensity of the color produced in the biuret reaction dependent?
    A. Molecular weight of the protein
    B. Acidity of the medium
    C. Number of peptide bonds
    D. Nitrogen content of the protein

81. Which of the following reagents can be used to measure protein in cerebrospinal fluid?
    A. Biuret
    B. Coomassie brilliant blue
    C. Ponceau S
    D. Bromcresol green

82. Which disorder is *not* associated with an elevated protein level in cerebrospinal fluid?
    A. Bacterial meningitis
    B. Multiple sclerosis
    C. Cerebral infarction
    D. Hyperthyroidism

83. Which term describes a congenital disorder that is characterized by a split in the albumin band when serum is subjected to electrophoresis?
    A. Analbuminemia
    B. Anodic albuminemia
    C. Prealbuminemia
    D. Bisalbuminemia

84. In what condition would an increased level of serum albumin be expected?
    A. Malnutrition
    B. Acute inflammation
    C. Dehydration
    D. Renal disease

85. Identification of which of the following is useful in early stages of glomerular dysfunction?
    A. Microalbuminuria
    B. Ketonuria
    C. Hematuria
    D. Urinary light chains

86. Which of the following is a low-weight protein that is found on the cell surfaces of nucleated cells?
    A. C-reactive protein
    B. β_2-Microglobulin
    C. Ceruloplasmin
    D. α_2-Macroglobulin

87. Which glycoprotein binds with hemoglobin to facilitate the removal of hemoglobin by the reticuloendothelial system?
    A. Haptoglobin
    B. Ceruloplasmin
    C. α_1-Antitrypsin
    D. Fibrinogen

88. In a healthy individual, which protein fraction has the greatest concentration in serum?
    A. Alpha$_1$-globulin
    B. Beta-globulin
    C. Gamma-globulin
    D. Albumin

89. Which of the following is an anionic dye that binds selectively with albumin?
    A. Amido black
    B. Ponceau S
    C. Bromcresol green
    D. Coomassie brilliant blue

90. Which total protein method requires copper sulfate, potassium iodide in sodium hydroxide, and potassium sodium tartrate in its reagent system?
    A. Kjeldahl
    B. Biuret
    C. Folin-Ciocalteu
    D. Ultraviolet absorption

91. Which of the following plasma proteins is *not* manufactured by the liver?
    A. Albumin
    B. Haptoglobin
    C. Fibrinogen
    D. IgG

92. There are five immunoglobulin classes: IgG, IgA, IgM, IgD, and IgE. With which globulin fraction do these immunoglobulins migrate electrophoretically?
    A. Alpha$_1$-globulins
    B. Alpha$_2$-globulins
    C. Beta$_1$-globulins
    D. Gamma-globulins

93. Of the five immunoglobulin classes, IgG is the most structurally simple, consisting of how many light chains/heavy chains, respectively?
    A. 5/2
    B. 1/1
    C. 2/5
    D. 2/2

94. Which immunoglobulin class, characterized by its possession of a secretory component, is found in saliva, tears, and body secretions?
    A. IgA
    B. IgD
    C. IgG
    D. IgM

95. Which immunoglobulin class is able to cross the placenta from the mother to the fetus?
    A. IgA
    B. IgD
    C. IgE
    D. IgG

96. Which of the following is an acute-phase reactant protein able to inhibit enzymatic proteolysis and having the highest concentration of any of the plasma proteolytic inhibitors?
    A. C-reactive protein
    B. Haptoglobin
    C. α_2-Macroglobulin
    D. α_1-Antitrypsin

97. Which of the following is a copper transport protein that migrates as an alpha$_2$-globulin?
    A. Ceruloplasmin
    B. Haptoglobin
    C. Transferrin
    D. Fibrinogen

98. Which of the following proteins is normally produced by the fetus but is found in increased amounts in the amniotic fluid in cases of spina bifida?
    A. α_1-Antitrypsin
    B. α_1-Acid glycoprotein
    C. α_1-Fetoprotein
    D. α_2-Macroglobulin

99. The physician is concerned that a pregnant patient may be at risk for delivering prematurely. What would be the best biochemical marker to measure to assess the situation?
    A. Inhibin A
    B. α_1-Fetoprotein
    C. Fetal fibronectin
    D. Human chorionic gonadotropin

100. Bence Jones proteinuria is a condition characterized by the urinary excretion of what type of light chain?
   A. Kappa light chains
   B. Lambda light chains
   C. Both kappa and lambda light chains
   D. Either kappa or lambda light chains

101. Which of the following is *not* characteristic of multiple myeloma?
   A. Monoclonal band in the gamma region
   B. Hypercalcemia
   C. Hyperalbuminemia
   D. Hyperglobulinemia

102. What technique is used to quantify specific immunoglobulin classes?
   A. Immunonephelometry
   B. Serum protein electrophoresis
   C. Isoelectric focusing
   D. Immunoelectrophoresis

103. Portal cirrhosis is a chronic disease of the liver. As observed on an electrophoretic serum protein pattern, what is a predominant characteristic of this disease?
   A. Monoclonal band in the gamma-globulin region
   B. Polyclonal band in the gamma-globulin region
   C. Bridging effect between the beta- and gamma-globulin bands
   D. Increase in the alpha$_2$-globulin band

104. The abnormal metabolism of several of the amino acids has been linked with disorders classified as inborn errors of metabolism. What technique is used to differentiate among several different amino acids?
   A. Electrophoresis
   B. Microbiological analysis
   C. Enzyme immunoassay
   D. Chromatography

105. Serum protein electrophoresis is routinely performed on the serum obtained from a clotted blood specimen. If a plasma specimen is substituted for serum, how will the electrophoresis be affected?
   A. Electrophoresis cannot be performed because the anticoagulant will retard the mobilities of the protein fractions.
   B. Electrophoresis cannot be performed because the anticoagulant will cause migration of the protein fractions in the direction of the cathode.
   C. Electrophoresis will show an extra fraction in the beta-gamma region.
   D. Electrophoresis will show an extra fraction in the prealbumin area.

106. In serum protein electrophoresis, when a barbital buffer of pH 8.6 is employed, what protein fraction will migrate the fastest toward the anode?
   A. Albumin
   B. Alpha$_1$-globulin
   C. Beta-globulin
   D. Gamma-globulin

107. In which of the following disorders would the maternal serum level of α_1-fetoprotein *not* be elevated?
   A. Neural tube defect
   B. Spina bifida
   C. Fetal distress
   D. Down syndrome

108. A male patient, 48 years old, mentions during his annual physical that he has been having difficulty urinating. The physician performs a rectal examination, and he orders a total prostate-specific antigen (PSA) and free PSA. The patient has the tests done the following week, and the total PSA result is 3.1 ng/mL and the free PSA is 0.3 ng/mL. What do these results suggest?
    A. Both are normal, no disease present
    B. Benign prostatic hypertrophy
    C. Increased risk of prostate cancer
    D. Free PSA is low and does not correlate with total PSA

109. Which of the following is *not* associated with carcinoembryonic antigen?
    A. Increased levels seen with malignancies of the lungs
    B. Quantified by using capillary electrophoresis
    C. Used to monitor treatment of colon cancer
    D. Glycoprotein in nature

110. In cases of hepatoma, which protein not normally found in adult serum is synthesized by liver cells?
    A. α_1-Acid glycoprotein
    B. α_1-Fetoprotein
    C. α_2-Macroglobulin
    D. Carcinoembryonic antigen

111. Which of the following is *false* about PSA?
    A. Serum quantified using immunoassays
    B. Single-chain glycoprotein
    C. Used as a tumor marker
    D. Not elevated in benign prostatic hyperplasia

112. Which of the following is an oncofetal antigen that is elevated in nonmucinous epithelial ovarian cancer?
    A. CA 549
    B. CA 125
    C. CA 19-9
    D. CA 15-3

113. Which of the following is a sialylated Lewis blood group antigen associated with colorectal carcinoma?
    A. CA 19-9
    B. CA 15-3
    C. CA 549
    D. CEA

114. Which of the following disorders is *not* associated with an elevation of serum α_1-fetoprotein?
    A. Testicular germ cell tumors
    B. Prostatic carcinoma
    C. Pancreatic carcinoma
    D. Gastric carcinoma

115. Which of the following is *not* associated with human chorionic gonadotropin?
    A. β subunit confers immunogenic specificity
    B. Used to confirm pregnancy
    C. Used as a tumor marker
    D. Found in hepatoma

116. Although serum elevations are not generally seen in early stages, which of the following tumor markers are elevated in more advanced stages of breast cancer?
    A. CEA and AFP
    B. AFP and CA 125
    C. PSA and CA 15-3
    D. CA 15-3 and CA 549

Nonprotein Nitrogenous Compounds

117. What is the compound that comprises the majority of the nonprotein-nitrogen fractions in serum?
   A. Uric acid
   B. Creatinine
   C. Ammonia
   D. Urea

118. Express 30 mg/dL of urea nitrogen as urea.
   A. 14 mg/dL
   B. 20 mg/dL
   C. 50 mg/dL
   D. 64 mg/dL

119. In the urea method, the enzymatic action of urease is inhibited when blood for analysis is drawn in a tube containing what anticoagulant?
   A. Sodium heparin
   B. Sodium fluoride
   C. Sodium oxalate
   D. Ethylenediaminetetra-acetic acid

120. In the diacetyl method, what does diacetyl react with to form a yellow product?
   A. Ammonia
   B. Urea
   C. Uric acid
   D. Nitrogen

121. What endogenous substance may cause a positive interference in the urease/glutamate dehydrogenase assay?
   A. Ammonia
   B. Creatinine
   C. Glucose
   D. Cholesterol

122. Which of the following methods utilizes urease and glutamate dehydrogenase for the quantification of serum urea?
   A. Berthelot
   B. Coupled enzymatic
   C. Conductimetric
   D. Indicator dye

123. In the Berthelot reaction, what contaminant will cause the urea level to be falsely elevated?
   A. Sodium fluoride
   B. Protein
   C. Ammonia
   D. Bacteria

124. To maintain acid-base balance, it is necessary that the blood ammonia level be kept within narrow limits. This is accomplished primarily by which of the following?
   A. Synthesis of urea from ammonia
   B. Synthesis of glutamine from ammonia
   C. Excretion of ammonia in the bile
   D. Excretion of ammonia in the stools

125. When a blood ammonia determination is performed, the blood specimen must be treated in a manner that will ensure that
   A. The deamination process continues *in vitro*
   B. Glutamine formation *in vitro* is avoided
   C. The transamination process continues *in vitro*
   D. Ammonia formation *in vitro* is avoided

126. Which of the following does *not* need to be done when collecting, handling, and using a specimen for ammonia analysis?
   A. Avoid using a hemolyzed specimen.
   B. Collect blood in EDTA or heparin evacuated tubes.
   C. Place specimen in a 37°C water bath immediately.
   D. Advise patient not to smoke for 8 hours before blood collection.

127. Which of the following statements can be associated with the enzymatic assay of ammonia?
   A. Increase in absorbance monitored at 340 nm
   B. Nicotinamide-adenine dinucleotide (NAD^+) required as a cofactor
   C. Ammonium ion isolated from specimen before the enzymatic step
   D. Reaction catalyzed by glutamate dehydrogenase

128. Which of the following disorders is *not* associated with an elevated blood ammonia level?
   A. Reye syndrome
   B. Renal failure
   C. Chronic liver failure
   D. Diabetes mellitus

129. An increased serum level of which of the following analytes is most commonly associated with decreased glomerular filtration?
   A. Creatinine
   B. Uric acid
   C. Urea
   D. Ammonia

130. A serum creatinine was found to be 6.0 mg/dL. Which of the following urea nitrogen serum results would support the same pathological condition?
   A. 6 mg/dL
   B. 20 mg/dL
   C. 35 mg/dL
   D. 70 mg/dL

131. From what precursor is creatinine formed?
   A. Urea
   B. Glucose
   C. Creatine
   D. Uric acid

132. What analyte is measured using the Jaffe reaction?
   A. Urea
   B. Uric acid
   C. Ammonia
   D. Creatinine

133. When the Jaffe reaction is employed as a kinetic assay to quantify serum creatinine, which of the following is used in the analysis?
   A. Serum sample used directly
   B. Folin-Wu filtrate
   C. Somogyi-Nelson filtrate
   D. Trichloroacetic acid filtrate

134. The creatinine clearance test is routinely used to assess the glomerular filtration rate. Given the following information for an average-size adult, calculate a creatinine clearance.
   Urine creatinine—120 mg/dL
   Plasma creatinine—1.2 mg/dL
   Urine volume for 24 hours—1520 mL
   A. 11 mL/min
   B. 63 mL/min
   C. 95 mL/min
   D. 106 mL/min

135. When it is not possible to perform a creatinine assay on a fresh urine specimen, to what pH level should the urine be adjusted?
   A. 3.0
   B. 5.0
   C. 7.0
   D. 9.0

136. What compound normally found in urine may be used to assess the completeness of a 24-hour urine collection?
   A. Urea
   B. Uric acid
   C. Creatine
   D. Creatinine

137. Which of the following reagents is *not* utilized in a coupled enzymatic reaction method to quantify serum creatinine?
A. Picric acid
B. Chromogenic dye
C. Creatinine amidohydrolase
D. Sarcosine oxidase

138. An endogenous substance assayed to assess the glomerular filtration rate may be described as being filtered by the glomeruli, not reabsorbed by the tubules, and only secreted by the tubules when plasma levels become elevated. What is this frequently assayed substance?
A. Inulin
B. Uric acid
C. Creatinine
D. Urea

139. What is the end product of purine catabolism in humans?
A. Urea
B. Uric acid
C. Allantoin
D. Ammonia

140. When mixed with phosphotungstic acid, what compound causes the reduction of the former to a tungsten blue complex?
A. Urea
B. Ammonia
C. Creatinine
D. Uric acid

141. In the ultraviolet procedure for quantifying uric acid, what does the reaction between uric acid and uricase cause?
A. Production of reduced nicotinamide-adenine dinucleotide (NADH)
B. The formation of allantoin
C. An increase in absorbance
D. A reduction of phosphotungstic acid

142. Which of the following disorders is best characterized by laboratory findings that include increased serum levels of inorganic phosphorus, magnesium, potassium, uric acid, urea, and creatinine and decreased serum calcium and erythropoietin levels?
A. Chronic renal failure
B. Renal tubular disease
C. Nephrotic syndrome
D. Acute glomerulonephritis

143. In gout, what analyte deposits in joints and other body tissues?
A. Calcium
B. Creatinine
C. Urea
D. Uric acid

144. During chemotherapy for leukemia, which of the following analytes would most likely be elevated in the blood?
A. Uric acid
B. Urea
C. Creatinine
D. Ammonia

Carbohydrates

145. What does hydrolysis of sucrose yield?
A. Glucose only
B. Galactose and glucose
C. Maltose and glucose
D. Fructose and glucose

146. In what form is glucose stored in muscle and liver?
A. Glycogen
B. Maltose
C. Lactose
D. Starch

147. Which of the following carbohydrates is a polysaccharide?
A. Starch
B. Sucrose
C. Lactose
D. Glucose

148. Which of the following defines the term "glycolysis"?
   A. Conversion of glucose into lactate or pyruvate
   B. Conversion of glucose to glycogen
   C. Breakdown of glycogen to form glucose
   D. Breakdown of lipids to form glucose

149. What is the glucose concentration in fasting whole blood?
   A. Less than the concentration in plasma or serum
   B. Greater than the concentration in plasma or serum
   C. Equal to the concentration in plasma or serum
   D. Meaningless because it is not stable

150. Of the following blood glucose levels, which would you expect to result in glucose in the urine?
   A. 60 mg/dL
   B. 120 mg/dL
   C. 150 mg/dL
   D. 225 mg/dL

151. Which test may be performed to assess the average plasma glucose level that an individual maintained during a previous 2- to 3-month period?
   A. Plasma glucose
   B. Two-hour postprandial glucose
   C. Oral glucose tolerance
   D. Glycated hemoglobin

152. The physician determined that the patient needed an oral glucose tolerance test (OGTT) to assist in diagnosis. The patient had blood drawn for the OGTT, and the following serum glucose results were obtained. These results are indicative of what state?

   Fasting serum glucose 124 mg/dL
   2-hour postload serum glucose 227 mg/dL

   A. Normal
   B. Diabetes mellitus
   C. Addison disease
   D. Hyperinsulinism

153. A 30-year-old pregnant woman has a gestational diabetes mellitus screening test performed at 26 weeks of gestation. Her physician chooses to order a 50-g oral glucose load. Her serum glucose level is 150 mg/dL at 1 hour. What should occur next?
   A. This confirms diabetes mellitus; give insulin.
   B. This confirms diabetes mellitus; dietary intake of carbohydrates should be lessened.
   C. This is suspicious of diabetes mellitus; an oral glucose tolerance test should be performed.
   D. This is an expected glucose level in a pregnant woman.

154. A sample of blood is collected for glucose in a sodium fluoride tube before the patient has had breakfast. The physician calls 2 hours later and requests that determination of blood urea nitrogen (BUN) be performed on the same sample rather than obtaining another specimen. The automated analyzer in your laboratory utilizes the urease method to quantify BUN. What should you tell the physician?
   A. Will gladly do the test if sufficient specimen remains
   B. Could do the test using a micromethod
   C. Can do the BUN determination on the automated analyzer
   D. Cannot perform the procedure

155. Which of the following does *not* properly describe type 1 diabetes mellitus?
   A. Insulin deficiency
   B. Associated with autoimmune destruction of pancreatic β-cells
   C. Ketoacidosis prone
   D. Occurs more frequently in adults

156. Which of the following is *not* associated with insulin?
   A. Synthesized from proinsulin
   B. Synthesized by β-cells in the pancreas
   C. C-peptide is active form
   D. Two-chain polypeptide

157. Which of the following statements may be associated with the activity of insulin?
   A. Increases blood glucose levels
   B. Decreases glucose uptake by muscle and fat cells
   C. Stimulates release of hepatic glucose into the blood
   D. Stimulates glycogenesis in the liver

158. Which of the following is *not* characteristic of severe hyperglycemia?
   A. Polyuria
   B. Ketonuria
   C. Glycosuria
   D. Hypoglucagonemia

159. Which of the following statements applies to the preferred use of plasma or serum, rather than whole blood, for glucose determination?
   A. Glucose is more stable in separated plasma or serum.
   B. Specificity for glucose is higher with most methods when plasma or serum is used.
   C. It is convenient to use serum or plasma with automated instruments because whole blood requires mixing immediately before sampling.
   D. All the above.

160. Which of the following analytes would *not* commonly be measured when monitoring complications of diabetes mellitus?
   A. Serum urea nitrogen
   B. Urinary albumin
   C. Serum creatinine
   D. Serum bilirubin

161. Ingestion of which of the following drugs may cause hypoglycemia?
   A. Ethanol
   B. Propranolol
   C. Salicylate
   D. All the above

162. Which of the following is *not* associated with hypoglycemia?
   A. Neuroglycopenia
   B. Symptoms occur with plasma glucose level of 60–70 mg/dL
   C. Decreased hepatic glucose production
   D. Diagnostic test is 72-hour fast

163. Which glucose method can employ a polarographic oxygen electrode?
   A. Hexokinase
   B. Glucose oxidase
   C. Glucose dehydrogenase
   D. *o*-Toluidine

164. Which glucose method catalyzes the phosphorylation of glucose by adenosine triphosphate, forming glucose-6-phosphate and adenosine diphosphate with the absorbance of the NADPH product read at 340 nm?
   A. *o*-Toluidine
   B. Glucose oxidase
   C. Hexokinase
   D. Glucose dehydrogenase

165. Which of the following is *not* a reagent required in an enzymatic serum glucose method?
A. NAD^+
B. Glucose oxidase
C. Peroxidase
D. Reduced chromogen

166. Which of the following glucose methods should *not* be used during the administration of an oral xylose absorption test?
A. Glucose oxidase—colorimetric
B. Glucose oxidase—polarographic
C. Glucose dehydrogenase
D. Hexokinase

167. Which glucose method is considered to be the reference method?
A. Glucose oxidase
B. *o*-Toluidine
C. Hexokinase
D. Glucose dehydrogenase

168. An individual has a plasma glucose level of 110 mg/dL. What would be the approximate glucose concentration in this patient's cerebrospinal fluid?
A. 33 mg/dL
B. 55 mg/dL
C. 66 mg/dL
D. 110 mg/dL

169. What is the reference interval for fasting serum glucose in an adult expressed in SI units (International System of Units)?
A. 1.7–3.3 mmol/L
B. 3.3–5.6 mmol/L
C. 4.1–5.5 mmol/L
D. 6.7–8.3 mmol/L

170. At what level should a 52-year-old male diagnosed with type 2 diabetes mellitus maintain his hemoglobin A_{1c}?
A. ≤3%
B. ≤7%
C. ≤9%
D. ≤11%

171. Which of the following hormones does *not* promote an increase in blood glucose levels?
A. Growth hormone
B. Cortisol
C. Glucagon
D. Insulin

172. What effect if any would be expected when the secretion of epinephrine is stimulated by physical or emotional stress?
A. Decreased blood glucose level
B. Increased blood glucose level
C. Increased glycogen storage
D. No effect on blood glucose or glycogen levels

173. What would an individual with Cushing syndrome tend to exhibit?
A. Hyperglycemia
B. Hypoglycemia
C. Normal blood glucose level
D. Decreased 2-hour postprandial glucose

174. As part of a routine physical, a fasting plasma glucose is performed on a 45-year-old male and the test result is 105 mg/dL. How should this individual be classified?
A. Normal for his age
B. Impaired fasting glucose
C. Type 1 diabetes mellitus
D. Type 2 diabetes mellitus

175. A cerebrospinal fluid specimen is sent to the lab at 9:00 P.M. for glucose analysis. The specimen is cloudy and appears to contain red blood cells. Which of the following statements is *true?*
A. Glucose testing cannot be performed on the specimen.
B. Specimen should be centrifuged and glucose assayed immediately.
C. Specimen can be refrigerated as received and glucose assayed the next day.
D. Specimen can be frozen as received and glucose assayed the next day.

176. A patient has a urine uric acid level of 1575 mg/day. What effect will this have on the measured urine glucose level when the glucose oxidase/peroxidase method is employed?
   A. Urine glucose level will be falsely low.
   B. Urine glucose level will be falsely high.
   C. Urine glucose level will be accurate.
   D. Urine glucose level will exceed the linearity of the method.

177. Laboratory tests are performed on a postmenopausal, 57-year-old female as part of an annual physical examination. The patient's casual plasma glucose is 220 mg/dL, and the glycated hemoglobin (Hb A_{1c}) is 11%. Based on this information, how should the patient be classified?
   A. Normal glucose tolerance
   B. Impaired glucose tolerance
   C. Gestational diabetes mellitus
   D. Type 2 diabetes mellitus

178. Which of the following is characterized by a deficiency of glucose-6-phosphatase resulting in hepatomegaly, lactic acidosis, and severe fasting hypoglycemia?
   A. Type I—von Gierke disease
   B. Type II—Pompe disease
   C. Type III—Cori disease
   D. Type IV—Andersen disease

Lipids and Lipoproteins

179. Bile acids that are synthesized in the liver are derived from what substance?
   A. Bilirubin
   B. Fatty acid
   C. Cholesterol
   D. Triglyceride

180. The turbid, or milky, appearance of serum after fat ingestion is termed postprandial lipemia, which is caused by the presence of what substance?
   A. Bilirubin
   B. Cholesterol
   C. Chylomicron
   D. Phospholipid

181. Cholesterol ester is formed through the esterification of the alcohol cholesterol with what substance?
   A. Protein
   B. Triglyceride
   C. Fatty acid
   D. Digitonin

182. Which of the following tests would most likely be included in a routine lipid profile?
   A. Total cholesterol, triglyceride, fatty acid, chylomicron
   B. Total cholesterol, triglyceride, HDL cholesterol, phospholipid
   C. Triglyceride, HDL cholesterol, LDL cholesterol, chylomicron
   D. Total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol

183. To produce reliable results, when should blood specimens for lipid studies be drawn?
   A. Immediately after eating
   B. Anytime during the day
   C. In the fasting state, approximately 2 to 4 hours after eating
   D. In the fasting state, approximately 9 to 12 hours after eating

184. Which of the following lipid tests is *least* affected by the fasting status of the patient?
   A. Cholesterol
   B. Triglyceride
   C. Fatty acid
   D. Lipoprotein

185. What compound is a crucial intermediary in the metabolism of triglyceride to form energy?
A. Bile
B. Acetyl-coenzyme A
C. Acetoacetate
D. Pyruvate

186. The kinetic methods for quantifying serum triglyceride employ enzymatic hydrolysis. The hydrolysis of triglyceride may be accomplished by what enzyme?
A. Amylase
B. Leucine aminopeptidase
C. Lactate dehydrogenase
D. Lipase

187. Enzymatic methods for the determination of total cholesterol in serum utilize a cholesterol oxidase-peroxidase method. In this method, cholesterol oxidase reacts specifically with what?
A. Free cholesterol and cholesteryl ester
B. Free cholesterol and fatty acid
C. Free cholesterol only
D. Cholesteryl ester only

188. Exogenous triglycerides are transported in the plasma in what form?
A. Phospholipids
B. Cholesteryl esters
C. Chylomicrons
D. Free fatty acids

189. Ketone bodies are formed because of an excessive breakdown of fatty acids. Of the following metabolites, which may be classified as a ketone body?
A. Pyruvic acid
B. β-Hydroxybutyric acid
C. Lactic acid
D. Oxaloacetic acid

190. Which of the following is most associated with the membrane structure of nerve tissue?
A. Cholesterol
B. Triglyceride
C. Phospholipids
D. Sphingolipids

191. Each lipoprotein fraction is composed of varying amounts of lipid and protein components. The beta-lipoprotein fraction consists primarily of which lipid?
A. Fatty acid
B. Cholesterol
C. Phospholipid
D. Triglyceride

192. What substance is the precursor to all steroid hormones?
A. Fatty acid
B. Cholesterol
C. Triglyceride
D. Phospholipid

193. The term "lipid storage diseases" is used to denote a group of lipid disorders, the majority of which are inherited as autosomal recessive mutations. What is the cause of these diseases?
A. Excessive dietary fat ingestion
B. Excessive synthesis of chylomicrons
C. A specific enzyme deficiency or nonfunctional enzyme form
D. An inability of adipose tissue to store lipid materials

194. Several malabsorption problems are characterized by a condition known as steatorrhea. Steatorrhea is caused by an abnormal accumulation of what substance in the feces?
A. Proteins
B. Lipids
C. Carbohydrates
D. Vitamins

195. What is the sedimentation nomenclature associated with alpha-lipoprotein?
A. Very-low-density lipoproteins (VLDLs)
B. High-density lipoproteins (HDLs)
C. Low-density lipoproteins (LDLs)
D. Chylomicrons

196. The quantification of the high-density lipoprotein cholesterol level is thought to be significant in the risk assessment of what disease?
A. Pancreatitis
B. Cirrhosis
C. Coronary artery disease
D. Hyperlipidemia

197. The surfactant/albumin ratio by fluorescence polarization is performed to assess what physiological state?
A. Hyperlipidemia
B. Coronary artery disease
C. Hemolytic disease of the newborn
D. Fetal lung maturity

198. The VLDL fraction primarily transports what substance?
A. Cholesterol
B. Chylomicron
C. Triglyceride
D. Phospholipid

199. A 54-year-old male, with a history of type 2 diabetes mellitus for the past 8 years, is seen by his family physician. The patient indicates that during the past week he had experienced what he described as feeling lightheaded and faint. He also indicated that he became out of breath and had experienced mild chest pain when doing heavy yard work, but the chest pain subsided when he sat down and rested. The physician performed an ECG immediately, which was normal, and he ordered blood tests. The patient fasted overnight and had blood drawn the next morning. The laboratory test values follow:

Test	Patient's Values	Reference Ranges
Glucose, fasting	175 mg/dL	74–99 mg/dL
Hemoglobin A_{1c}	8.1%	4–6%
Total cholesterol	272 mg/dL	<200mg/dL
HDL cholesterol	30 mg/dL	>40 mg/dL
LDL cholesterol	102 mg/dL	<130 mg/dL
Triglyceride	250 mg/dL	<150 mg/dL
hs-CRP	6.2 mg/L	0.3–8.6 mg/L, <1.0 mg/L low risk

Based on the patient's test results, history, and symptoms, which of the laboratory values in the chart above does *not* support the patient's diagnosis?
A. LDL cholesterol
B. HDL cholesterol
C. Hemoglobin A_{1c}
D. hs-CRP

200. Name a commonly used precipitating reagent to separate HDL cholesterol from other lipoprotein cholesterol fractions.
A. Zinc sulfate
B. Trichloroacetic acid
C. Heparin-manganese
D. Isopropanol

201. What is the principle of the "direct" or "homogeneous" HDL cholesterol automated method, which requires no intervention by the laboratorian? The direct HDL method
   A. Quantifies only the cholesterol in HDL, whereas the precipitation HDL method quantifies the entire lipoprotein
   B. Utilizes polymers and detergents that make the HDL cholesterol soluble while keeping the other lipoproteins insoluble
   C. Uses a nonenzymatic method to measure cholesterol, whereas the other methods use enzymes to measure cholesterol
   D. Uses a column chromatography step to separate HDL from the other lipoproteins, whereas the other methods use a precipitation step

202. Which of the following results would be the most consistent with high risk for coronary heart disease?
   A. 20 mg/dL HDL cholesterol and 250 mg/dL total cholesterol
   B. 45 mg/dL HDL cholesterol and 210 mg/dL total cholesterol
   C. 50 mg/dL HDL cholesterol and 180 mg/dL total cholesterol
   D. 55 mg/dL HDL cholesterol and 170 mg/dL total cholesterol

203. A patient's total cholesterol is 300 mg/dL, his HDL cholesterol is 50 mg/dL, and his triglyceride is 200 mg/dL. What is this patient's calculated LDL cholesterol?
   A. 200
   B. 210
   C. 290
   D. 350

204. A patient's total cholesterol/HDL cholesterol ratio is 10.0. What level of risk for coronary heart disease does this result indicate?
   A. No risk
   B. Half average risk
   C. Average risk
   D. Twice average risk

205. Which of the following techniques can be used to quantify apolipoproteins?
   A. Spectrophotometric endpoint
   B. Ion-selective electrode
   C. Immunonephelometric assay
   D. Refractometry

206. Which of the following may be described as a variant form of LDL, associated with increased risk of atherosclerotic cardiovascular disease?
   A. Lp(a)
   B. HDL
   C. Apo A-I
   D. Apo A-II

207. In what way is the "normal" population reference interval for total cholesterol in America different from that of other clinical chemistry parameters (i.e., protein, sodium, BUN, creatinine, etc.)?
   A. Established units for total cholesterol are mg/dL; no other chemistry test has these units.
   B. Reference interval is artificially set to reflect good health even though Americans as a group have "normally" higher total cholesterol levels.
   C. Total cholesterol reference interval must be interpreted in line with triglyceride, phospholipid, and sphingolipid values.
   D. Total cholesterol reference interval is based on a manual procedure, whereas all other chemistry parameters are based on automated procedures.

208. Your lab routinely uses a precipitation method to separate HDL cholesterol. You receive a slightly lipemic specimen for HDL cholesterol. The total cholesterol and triglyceride for the specimen were 450 and 520 mg/dL, respectively. After adding the precipitating reagents and centrifuging, you notice that the supernatant still looks slightly cloudy. What is your next course of action in analyzing this specimen?
   A. Perform the HDL cholesterol test; there is nothing wrong with this specimen.
   B. Take off the supernatant and recentrifuge.
   C. Take off the supernatant and add another portion of the precipitating reagent to it and recentrifuge.
   D. Send specimen to a lab that offers other techniques to separate more effectively the HDL cholesterol.

209. A 46-year-old known alcoholic with liver damage is brought into the emergency department unconscious. In what way would you expect his plasma lipid values to be affected?
   A. Increased total cholesterol, triglyceride, LDL, and VLDL
   B. Increased total cholesterol and triglyceride, decreased LDL and VLDL
   C. Decreased total cholesterol, triglyceride, LDL, and VLDL
   D. Normal lipid metabolism, unaffected by the alcoholism

210. A healthy, active 10-year-old boy with no prior history of illness comes to the lab after school for a routine chemistry screen in order to meet requirements for summer camp. After centrifugation, the serum looks cloudy. The specimen had the following results: blood glucose = 135 mg/dL, total cholesterol = 195 mg/dL, triglyceride = 185 mg/dL. What would be the most probable explanation for these findings? The boy
   A. Is at risk for coronary artery disease
   B. Has type 1 diabetes mellitus that is undiagnosed
   C. Has an inherited genetic disease causing a lipid imbalance
   D. Was most likely not fasting when the specimen was drawn

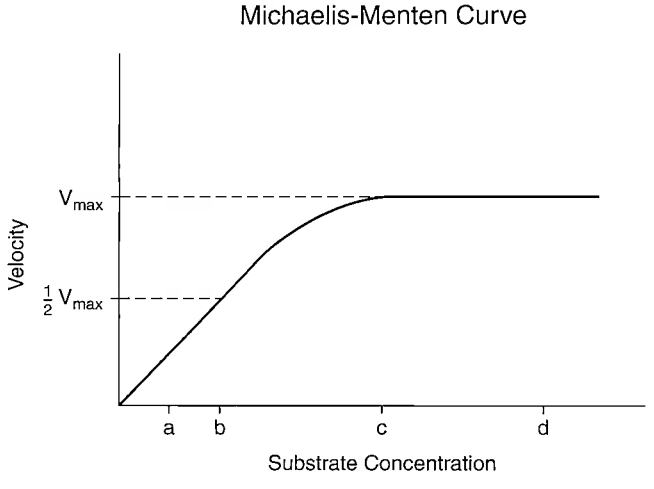
211. A mother brings her obese, 4-year-old child who is a known type 1 diabetic to the laboratory for a blood workup. She states that the boy has been fasting for the past 12 hours. After centrifugation the tech notes that the serum looks turbid. The specimen had the following results: blood glucose = 150 mg/dL, total cholesterol = 250 mg/dL, HDL cholesterol = 32 mg/dL, triglyceride = 395 mg/dL. What best explains these findings? The boy
   A. Is a low risk for coronary artery disease
   B. Is a good candidate for a 3-hour oral glucose tolerance test
   C. Has secondary hyperlipidemia due to the diabetes
   D. Was not fasting when the specimen was drawn

Enzymes and Cardiac Assessment

212. What does an increase in the serum enzyme levels indicate?
   A. Decreased enzyme catabolism
   B. Accelerated enzyme production
   C. Tissue damage and necrosis
   D. Increased glomerular filtration rate

213. In the assay of an enzyme, zero-order kinetics are best described by which of the following statements?
   A. Enzyme is present in excess; rate of reaction is variable with time and dependent only on the concentration of the enzyme in the system.
   B. Substrate is present in excess; rate of reaction is constant with time and dependent only on the concentration of enzyme in the system.
   C. Substrate is present in excess; rate of reaction is constant with enzyme concentration and dependent only on the time in which the reaction is run.
   D. Enzyme is present in excess; rate of reaction is independent of both time and concentration of the enzyme in the system.

214. Based on the following graph of velocity of an enzyme reaction versus substrate concentration, you are designing a new method to measure the activity of an enzyme of clinical interest. To formulate the new methodology so that enzyme activity is assessed using zero-order kinetics, which concentration of substrate should you initially determine experimentally?
   A. Substrate concentration *a*
   B. Substrate concentration *b*
   C. Substrate concentration *c*
   D. Substrate concentration *d*



215. When measuring enzyme activity, if the instrument is operating 5°C lower than the temperature prescribed for the method, how will the results be affected?
   A. Lower than expected
   B. Higher than expected
   C. Varied, showing no particular pattern
   D. All will be clinically abnormal.

216. Given the following information for a rate reaction, calculate the activity of a serum specimen for alanine aminotransferase in international units per liter (IU/L).

Time	Absorbance	
1 min	1.104	Specimen volume = 20 µL
2 min	1.025	Reagent volume = 3.0 mL
3 min	0.950	Molar absorptivity for NADH at 340 nm = 6.22×10^3 L/mol·cm
4 min	0.873	Light path = 1 cm

   A. 186
   B. 198
   C. 1857
   D. 1869

217. The properties of enzymes are correctly described by which of the following statements?
   A. Enzymes are stable proteins.
   B. Enzymes are protein catalysts of biological origin.
   C. Enzymes affect the rate of a chemical reaction by raising the activation energy needed for the reaction to take place.
   D. Enzyme activity is not altered by heat denaturation.

218. Which of the following is a *true* statement concerning serum enzymes?
   A. The presence of hemolyzed red cells is of no significance for an accurate assay of most serum enzymes.
   B. Serum asparate transaminase (AST), but not serum lactate dehydrogenase (LD), is usually elevated in acute myocardial infarction.
   C. Increased serum alkaline phosphatase may be found in bone disease.
   D. Aspartate transaminase was formerly known as glutamate pyruvate transaminase.

219. Enzymes that catalyze the transfer of groups between compounds are classified as belonging to which enzyme class?
   A. Hydrolases
   B. Lyases
   C. Oxidoreductases
   D. Transferases

220. Which of the following enzymes does *not* belong to the class of enzymes known as the hydrolases?
   A. Alkaline phosphatase
   B. Aldolase
   C. Amylase
   D. Lipase

221. To what class of enzymes does lactate dehydrogenase belong?
   A. Isomerases
   B. Ligases
   C. Oxidoreductases
   D. Transferases

222. Which of the following enzymes catalyzes the transfer of amino groups causing the interconversion of amino acids and α-oxoacids?
   A. Amylase
   B. Aspartate transaminase
   C. Alkaline phosphatase
   D. Lactate dehydrogenase

223. What abbreviation has been used in the past to designate alanine aminotransferase?
   A. AST
   B. AAT
   C. GOT
   D. GPT

224. When measuring CK-MB, which of the following would provide the most sensitive method?
   A. Electrophoretic
   B. Colorimetric
   C. Kinetic
   D. Mass immunoassay

225. Which of the following does *not* accurately describe properties associated with lactate dehydrogenase?
   A. Optimum pH for the catalysis of lactate to pyruvate is 7.4–7.8.
   B. LD is increased in a hemolyzed serum specimen.
   C. LD catalyzes the oxidation of lactate to pyruvate with mediation of nicotinamide-adenine dinucleotide.
   D. LD-4 and LD-5 are labile in the cold.

226. Which test, if elevated, would provide information about risk for developing coronary artery disease?
   A. Troponin
   B. CK-MB
   C. hs-CRP
   D. Myoglobin

227. Lactate dehydrogenase (LD) catalyzes the following reaction:

$$\text{Lactate} + \text{NAD}^+ \overset{\text{LD}}{\rightleftharpoons} \text{pyruvate} + \text{NADH}$$

   As the reaction is written, which of the following techniques can be used to assess LD activity?
   A. Measure the colorimetric product pyruvate.
   B. Measure the colorimetric product NADH.
   C. Measure the increase in absorbance at 340 nm as NADH is produced.
   D. Measure the decrease in absorbance at 340 nm as NADH is produced.

228. Which of the following is *false* about myoglobin as it relates to acute myocardial infarction (AMI)?
   A. Measure serially
   B. Cardiac specific
   C. Initial increase occurs in 1–3 hours
   D. Doubling of initial value within 1–2 hours suggestive of AMI

229. Which of the following disorders is *not* associated with an elevation of serum creatine kinase?
   A. Cerebrovascular accidents
   B. Hypothyroidism
   C. Bone disease
   D. Intramuscular injection

230. Which of the following statements concerning creatine kinase is *false*?
   A. Rises within 4–6 hours after acute myocardial infarction
   B. Catalyzes the phosphorylation of creatine by ATP
   C. Requires Ca^{2+} for activity
   D. Found mainly in skeletal and cardiac muscles and in brain tissue

231. Which enzyme is measured by methodologies that use small oligosaccharides and 4-nitrophenyl-glycoside for substrates?
   A. Lipase
   B. Amylase
   C. Creatine kinase
   D. Cholinesterase

232. Which statement concerning gamma-glutamyltransferase is *false?*
   A. Present in almost all cells of the body
   B. Elevated in liver and some pancreatic diseases
   C. Elevated in chronic alcoholism
   D. Elevated in bone disease

233. Which of the following statements correctly describes alkaline phosphatase?
   A. Decreased in Paget disease
   B. Decreased in third trimester of a normal pregnancy
   C. Increased in obstructive jaundice
   D. Primarily found in cardiac muscle

234. Which of the following enzymes would *not* be useful to quantify in the assessment of liver function?
   A. Alanine aminotransferase
   B. Creatine kinase
   C. Alkaline phosphatase
   D. Gamma-glutamyltransferase

235. In acute pancreatitis, a significant increase in which serum enzyme would be expected diagnostically?
   A. Creatine kinase
   B. Amylase
   C. Alkaline phosphatase
   D. Aspartate aminotransferase

236. For assessing carcinoma of the prostate, quantification of PSA has virtually replaced the measurement of which of the following enzymes?
   A. Alkaline phosphatase
   B. Acid phosphatase
   C. Alanine aminotransferase
   D. Trypsin

237. Which of the following statements is *not* associated with serum cholinesterase?
   A. Inhibited by organic insecticides
   B. Referred to as "true" cholinesterase
   C. Decreased level causes prolonged apnea after administration of succinyldicholine
   D. Acts on the substrate propionylthiocholine

238. Which of the following disorders is *not* characterized by an elevated serum myoglobin?
   A. Renal failure
   B. Vigorous exercise
   C. Acute myocardial infarction
   D. Hepatitis

239. Which of the following is *false* about cardiac troponin I (cTnI) as it relates to AMI?
   A. Increase above reference interval seen in 3 to 6 hours
   B. Measure initially and serially in 3- to 6-hour intervals
   C. Remains elevated 5 to 10 days
   D. Expressed in regenerating and diseased skeletal muscle and cardiac muscle disorders

240. Which of the following sets of tests would be the most useful in diagnosing an AMI?
   A. AST, LD, CK-MB
   B. LD, CK-MB, troponin
   C. CK-MB, troponin, myoglobin
   D. LD, troponin, myoglobin

241. A physician orders several laboratory tests on a 55-year-old male patient who is complaining of pain, stiffness, fatigue, and headaches. Based on the following serum test results, what is the most likely diagnosis?
   Alkaline phosphatase—significantly increased
   Gamma-glutamyltransferase—normal
   A. Biliary obstruction
   B. Cirrhosis
   C. Hepatitis
   D. Osteitis deformans

242. A 53-year-old female presents with fatigue, pruritus, and an enlarged, nontender liver. The physician orders a series of blood tests. Based on the following serum test results, what is the most likely diagnosis?
   Alkaline phosphatase—markedly elevated
   Alanine aminotransferase—slightly elevated
   Lactate dehydrogenase—slightly elevated
   Gamma-glutamyltransferase—markedly elevated
   Total bilirubin—slightly elevated
   A. Alcoholic cirrhosis
   B. Infectious mononucleosis
   C. Intrahepatic cholestasis
   D. Viral hepatitis

243. A 42-year-old male presents with anorexia, nausea, fever, and icterus of the skin and mucous membranes. He noticed that his urine had appeared dark for the past several days. The physician orders a series of biochemical tests. Based on the following test results, what is the most likely diagnosis?
    Serum alkaline phosphatase—slightly elevated
    Serum alanine aminotransferase—markedly elevated
    Serum aspartate aminotransferase—markedly elevated
    Serum gamma-glutamyltransferase—slightly elevated
    Serum total bilirubin—moderately elevated
    Urine bilirubin—positive
    Fecal urobilinogen—decreased
    A. Acute hepatitis
    B. Alcoholic cirrhosis
    C. Metastatic carcinoma of the pancreas
    D. Obstructive jaundice

244. To aid in the diagnosis of skeletal muscle disease, which of the following serum enzyme measurements would be of most use?
    A. Creatine kinase
    B. Alkaline phosphatase
    C. Aspartate aminotransferase
    D. Alanine aminotransferase

245. When an AMI occurs, in what order (list first to last) will the enzymes aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LD) become elevated in the serum?
    A. AST, LD, CK
    B. CK, LD, AST
    C. CK, AST, LD
    D. LD, CK, AST

246. Which of the following is *not* associated with assessment of an AMI?
    A. Elevated serum cTnI level
    B. Elevated serum CK-MB level
    C. Abnormal serum alkaline phosphatase isoenzyme pattern
    D. Blood collected upon presentation and serially in 3- to 6-hour intervals

247. If elevated, which laboratory test would support a diagnosis of congestive heart failure?
    A. Homocysteine
    B. Troponin
    C. Albumin cobalt binding
    D. B-type natriuretic peptide

248. A 4-year-old male child is brought to the pediatrician because the parents are concerned about the child's frequent falling, which results in bruising. The parents indicate that the child has difficulty running, walking, standing up, climbing stairs, and even sitting up straight. The child also appears somewhat weak. Which of the following results is *not* consistent with the most likely diagnosis?
    A. Moderately elevated AST
    B. Moderately elevated ALP
    C. Moderately elevated LD
    D. Markedly elevated CK

249. A 68-year-old male in an unconscious state is transported to the emergency department after being involved in a one-car crash, where he drove off the road and hit a tree. Because he was alone at the time and there was no apparent cause for the accident, it is assumed that he blacked out, which caused him to lose control of the car. He was not wearing a seat belt and has a broken leg, multiple contusions, and cuts. Blood samples were drawn upon arrival to the ED and in 3-hour intervals for 12 hours; all control values were within acceptable range. Selected test results follow:

Test	Initial Values	3 Hours	9 Hours	Reference Ranges
Myoglobin	57 ng/mL	140 ng/mL	281 ng/mL	30–90 ng/mL
Total CK	112 U/L	170 U/L	390 U/L	15–160 U/L
CK-MB	3 ng/mL	6 ng/mL	8 ng/mL	0–5 ng/mL
Troponin I	0.10 ng/mL	0.12 ng/mL	0.11 ng/mL	<0.40 ng/mL

What do these test results suggest?

A. The man had a myocardial infarction, which caused the accident.
B. The elevated results are from the skeletal muscle injuries sustained in the car crash.
C. The elevated results are a combination of the car crash injuries and a myocardial infarction.
D. The elevated total CK and CK-MB results indicate that the man had a stroke.

250. If elevated, which of the following is associated with increased risk for coronary heart disease?

A. Homocysteine
B. Vitamin B_6
C. Myoglobin
D. pro-BNP

251. Which statement best describes the clinical use of measuring NT-proBNP?

A. Used to assess risk of coronary heart disease
B. Used to assess risk of angina
C. Used to assess individuals treated with nesiritide
D. Used to assess individuals treated with vitamin B

252. A 10-year-old female presents with varicella. The child has been experiencing fever, nausea, vomiting, lethargy, and disorientation. A diagnosis of Reye syndrome is determined. Which of the following laboratory results is *not* consistent with the diagnosis?

A. Elevated serum AST
B. Elevated serum ALT
C. Elevated plasma ammonia
D. Elevated serum bilirubin

253. Which of the following enzyme activities can be determined by using a dilute olive oil emulsion substrate, whose hydrolyzed product is monitored as a decrease in turbidity or light scatter?
   A. Alkaline phosphatase
   B. Amylase
   C. Lipase
   D. Trypsin

254. Which of the following is *not* characteristic of cystic fibrosis?
   A. Decreased bicarbonate concentration in duodenal fluid
   B. Decreased lipase activity in duodenal fluid
   C. Decreased amylase activity in duodenal fluid
   D. Increased trypsin in feces

Liver Function and Porphyrin Formation

255. Which compounds originally condense to form aminolevulinic acid?
   A. Oxoglutarate and aspartate
   B. Isocitrate and coenzyme II
   C. Oxalacetate and malate
   D. Succinyl coenzyme A and glycine

256. What compound chelates iron and is the immediate precursor of heme formation?
   A. Porphobilinogen
   B. Protoporphyrinogen IX
   C. Uroporphyrinogen III
   D. Protoporphyrin IX

257. Which of the following is a qualitative screening test for porphobilinogen that may be performed to aid in the diagnosis of the porphyrias?
   A. Caraway test
   B. Gutman test
   C. Jendrassik-Grof test
   D. Watson-Schwartz test

258. What compound may be detected by observing its orange-red fluorescence in acid solution?
   A. Porphobilinogen
   B. Uroporphyrinogen
   C. Aminolevulinic acid
   D. Coproporphyrin

259. The laboratory receives a request that assays for urinary aminolevulinic acid, porphobilinogen, uroporphyrin, and coproporphyrin are to be performed on a patient. Which of the following will *not* contribute to the integrity of the sample when these assays are performed on the same urine specimen?
   A. Refrigeration
   B. Addition of hydrochloric acid
   C. 24-hour urine collection
   D. Use of a brown bottle

260. What is the immediate precursor of bilirubin formation?
   A. Mesobilirubinogen
   B. Verdohemoglobin
   C. Urobilinogen
   D. Biliverdin

261. To quantify serum bilirubin levels, it is necessary that bilirubin couples with diazotized sulfanilic acid to form what complex?
   A. Verdobilirubin
   B. Azobilirubin
   C. Azobilirubinogen
   D. Bilirubin glucuronide

262. What enzyme catalyzes the conjugation of bilirubin?
   A. Leucine aminopeptidase
   B. Glucose-6-phosphate dehydrogenase
   C. Uridine diphosphate glucuronyltransferase
   D. Carbamoyl phosphate synthetase

263. What breakdown product of bilirubin metabolism is produced in the colon from the oxidation of urobilinogen by microorganisms?
A. Porphobilinogen
B. Urobilin
C. Stercobilinogen
D. Protoporphyrin

264. Which of the following functions as a transport protein for bilirubin in the blood?
A. Albumin
B. Alpha$_1$-globulin
C. Beta-globulin
D. Gamma-globulin

265. What term is used to describe the accumulation of bilirubin in the skin?
A. Jaundice
B. Hemolysis
C. Cholestasis
D. Kernicterus

266. In the condition kernicterus, the abnormal accumulation of bilirubin occurs in what tissue?
A. Brain
B. Liver
C. Kidney
D. Blood

267. As a reduction product of bilirubin catabolism, this compound is partially reabsorbed from the intestine through the portal circulation for reexcretion by the liver. What is this compound?
A. Verdohemoglobin
B. Urobilinogen
C. Urobilin
D. Biliverdin

268. Which of the following factors will *not* adversely affect the accurate quantification of bilirubin in serum?
A. Lipemia
B. Hemolysis
C. Exposure to light
D. Specimen refrigeration

269. Which bilirubin fraction is unconjugated and covalently bound to albumin?
A. Alpha
B. Beta
C. Delta
D. Gamma

270. As the red blood cells disintegrate, hemoglobin is released and converted to the pigment bilirubin. Which organ is primarily responsible for this function?
A. Spleen
B. Kidneys
C. Intestines
D. Liver

271. Which of the following methods is *not* used for the quantification of serum bilirubin?
A. Bilirubinometer
B. Jendrassik and Grof
C. Zimmerman
D. Bilirubin oxidase

272. Which of the following does *not* accurately describe direct bilirubin?
A. Insoluble in water
B. Conjugated in the liver
C. Conjugated with glucuronic acid
D. Excreted in the urine of jaundiced patients

273. Which of the following reagent systems contains the components sulfanilic acid, hydrochloric acid, and sodium nitrite?
A. Jaffe
B. Zimmerman
C. Diazo
D. Lowry

274. Indirect-reacting bilirubin may be quantified by reacting it initially in which reagent?
   A. Dilute hydrochloric acid
   B. Dilute sulfuric acid
   C. Caffeine-sodium benzoate
   D. Sodium hydroxide

275. Which of the following methods employs a reaction where bilirubin is oxidized to colorless biliverdin?
   A. Bilirubinometer
   B. Bilirubin oxidase
   C. High-performance liquid chromatography
   D. Jendrassik-Grof

276. What collective term encompasses the reduction products stercobilinogen, urobilinogen, and mesobilirubinogen?
   A. Urobilinogen
   B. Mesobilirubinogen
   C. Urobilin
   D. Bilirubin

277. What condition is characterized by an elevation of total bilirubin primarily due to an increase in the conjugated bilirubin fraction?
   A. Hemolytic jaundice
   B. Neonatal jaundice
   C. Crigler-Najjar syndrome
   D. Obstructive jaundice

278. Which of the following is characteristic of hemolytic jaundice?
   A. Unconjugated serum bilirubin level increased
   B. Urinary bilirubin level increased
   C. Urinary urobilinogen level decreased
   D. Fecal urobilin level decreased

279. What may be the cause of neonatal physiological jaundice of the hepatic type?
   A. Hemolytic episode caused by an ABO incompatibility
   B. Stricture of the common bile duct
   C. Hemolytic episode caused by an Rh incompatibility
   D. Deficiency in the bilirubin conjugation enzyme system

280. Which of the following laboratory results is *not* characteristic of a complete obstruction of the common bile duct?
   A. Negative urine urobilinogen
   B. Negative fecal urobilinogen and urobilin
   C. Negative urine bilirubin
   D. Excretion of a pale-colored stool

281. Which of the following characterizes hepatic dysfunction in the early stage of viral hepatitis?
   A. Elevation in urobilinogen and urobilin excretion in the feces
   B. Elevation in the serum unconjugated bilirubin fraction
   C. Depression in the serum conjugated bilirubin fraction
   D. Depression in urinary urobilinogen excretion

282. Which of the following characterizes Crigler-Najjar syndrome?
   A. Inability to transport bilirubin from the sinusoidal membrane to the microsomal region
   B. Deficiency of the enzyme system required for conjugation of bilirubin
   C. Inability to transport bilirubin glucuronides to the bile canaliculi
   D. Severe liver cell damage accompanied by necrosis

283. Which of the following disorders is characterized by an inability to transport bilirubin from the sinusoidal membrane into the hepatocyte?
A. Carcinoma of the common bile duct
B. Crigler-Najjar syndrome
C. Dubin-Johnson syndrome
D. Gilbert syndrome

284. Which of the following is *not* characteristic of Dubin-Johnson syndrome?
A. Impaired excretion of bilirubin into the bile
B. Hepatic uptake of bilirubin is normal
C. Inability to conjugate bilirubin
D. Increased level of bilirubin in urine

285. Which of the following disorders is *not* a form of hepatic jaundice?
A. Cirrhosis
B. Crigler-Najjar syndrome
C. Hepatitis
D. Neoplasm of common bile duct

286. Which of the following disorders can be classified as a form of prehepatic jaundice?
A. Acute hemolytic anemia
B. Cirrhosis
C. Dubin-Johnson syndrome
D. Neoplasm of common bile duct

287. The following laboratory results are determined on a patient with a suggested diagnosis of biliary obstruction:
Serum total bilirubin—increased
Serum conjugated bilirubin—normal
Urine bilirubin—increased
Fecal urobilin—decreased
Which laboratory result is the *least* consistent with such a diagnosis?
A. Serum total bilirubin
B. Serum conjugated bilirubin
C. Urine bilirubin
D. Fecal urobilin

288. A 42-year-old woman is admitted to the hospital with complaints of abdominal pain and inability to eat, which have gotten worse during the past several weeks. Although the pain had been uncomfortable, what alarmed her was noticing a slight yellow color in her eyes. Blood was drawn and the test results follow: total bilirubin 3.9 mg/dL, direct bilirubin 2.7 mg/dL, AST slightly elevated (3 times the upper limit of the reference range), ALT slightly elevated (3 times the upper limit of the reference range), alkaline phosphatase markedly elevated (6 times the upper limit of the reference range), and urine urobilinogen decreased. What diagnosis do these test results support?
A. Viral hepatitis
B. Cirrhosis
C. Exposure to toxic chemicals
D. Biliary obstruction

289. Which of the following results is *least* consistent with a diagnosis of viral hepatitis?
A. Serum total bilirubin 7.5 mg/dL, direct bilirubin 5.5 mg/dL, indirect bilirubin 2.0 mg/dL
B. Urine urobilinogen increased
C. AST increased 10 times the upper limit of the reference range
D. ALT increased 13 times the upper limit of the reference range

Electrolytes and Osmolality

290. What is the normal renal threshold of sodium (measured in millimoles per liter)?
A. 80–85
B. 90–110
C. 110–130
D. 135–148

291. Of the total serum osmolality, sodium, chloride, and bicarbonate ions normally contribute approximately what percent?
A. 8
B. 45
C. 75
D. 92

292. The presence of only slightly visible hemolysis will significantly increase the serum level of which of the following electrolytes?
A. Sodium
B. Potassium
C. Chloride
D. Bicarbonate

293. Which of the following is *not* a component of the total anion content of serum?
A. Acetoacetate
B. Protein
C. Lactate
D. Iron

294. Which of the following is *not* associated with potassium?
A. Has no renal threshold
B. Increased serum level in acidosis
C. Hemolysis causes false increase in serum levels
D. Major anion of intracellular fluid

295. Which of the following is a spectrophotometric method for quantifying serum chloride?
A. Ferric perchlorate
B. Ammonium molybdate
C. Bathophenanthroline
D. Cresolphthalein complexone

296. Which of the following statements is *false* about the electrolyte chloride?
A. Main anion of the extracellular fluid
B. Can shift from the extracellular plasma to the intracellular fluid of red blood cells
C. Unable to be reabsorbed by active transport
D. Measured in serum, urine, and sweat

297. Using the following data: $Na^+ = 143$ mmol/L; $K^+ = 4.9$ mmol/L; $Cl^- = 105$ mmol/L; and $HCO_3^- = 25$ mmol/L, which of the following statements is *false?*
A. Patient results are not acceptable.
B. Anion gap is useful in detecting some disease states.
C. Anion gap equals 18 mmol/L.
D. Anion gap is useful for checking analytical error.

298. A patient presents with Addison disease. Serum sodium and potassium analyses are performed. What would the results reveal?
A. Normal sodium, low potassium levels
B. Low sodium, low potassium levels
C. Low sodium, high potassium levels
D. High sodium, low potassium levels

299. Primary aldosteronism results from a tumor of the adrenal cortex. How would the extracellular fluid be affected?
A. Normal sodium, decreased potassium levels
B. Decreased sodium, decreased potassium levels
C. Decreased sodium, increased potassium levels
D. Increased sodium, decreased potassium levels

300. Which of the following conditions is *not* associated with hyponatremia?
A. Addison disease
B. Diarrhea
C. Diuretic therapy
D. Cushing syndrome

301. Of the total serum calcium, free ionized calcium normally represents approximately what percent?
A. 10
B. 40
C. 50
D. 90

302. Measuring the tubular reabsorption of phosphate is useful in diagnosing diseases that affect which of the following organs?
A. Liver
B. Adrenal gland
C. Thyroid gland
D. Parathyroid gland

303. Which of the following does *not* have an effect on plasma calcium levels?
A. Parathyroid hormone
B. Vitamin D
C. Calcitonin
D. Aldosterone

304. Which of the following is an effect of increased parathyroid hormone secretion?
A. Decreased blood calcium levels
B. Increased renal reabsorption of phosphate
C. Decreased bone resorption
D. Increased intestinal absorption of calcium

305. The following laboratory results are obtained on a 60-year-old woman who is complaining of anorexia, constipation, abdominal pain, nausea, and vomiting:
Ionized serum calcium—elevated
Serum inorganic phosphate—decreased
Urine calcium—elevated
Urine phosphate—elevated
What do these results suggest?
A. Primary hyperparathyroidism
B. Vitamin D deficiency
C. Hypoparathyroidism
D. Paget disease

306. Secondary hyperparathyroidism is often the result of
A. Vitamin C deficiency
B. Liver disease
C. Renal disease
D. Thyroid disease

307. Which of the following reagents is used to determine the concentration of serum inorganic phosphate?
A. Ehrlich's reagent
B. Ammonium molybdate
C. 8-Hydroxyquinoline
D. Bathophenanthroline

308. Which of the following reagents is used in a colorimetric method to quantify the concentration of serum calcium?
A. Cresolphthalein complexone
B. Lanthanum
C. Malachite green
D. Amino-naphthol-sulfonic acid

309. Which of the following has an effect on plasma calcium levels?
A. Sodium
B. Inorganic phosphate
C. Potassium
D. Iron

310. A patient's serum inorganic phosphate level is found to be elevated but the physician cannot determine a physiological basis for this abnormal result. What could possibly have caused an erroneous result to be reported?
    A. Patient not fasting when blood was drawn
    B. Specimen was hemolyzed
    C. Effect of diurnal variation
    D. Patient receiving intravenous glucose therapy

311. To what metal does ceruloplasmin firmly bind?
    A. Chromium
    B. Copper
    C. Zinc
    D. Iron

312. In iron-deficiency anemia, what would be the expected percent saturation of transferrin with iron?
    A. Less than 15
    B. Between 30 and 40
    C. Between 40 and 50
    D. Greater than 55

313. What is the primary storage form of iron?
    A. Apotransferrin
    B. Myoglobin
    C. Ferritin
    D. Hemosiderin

314. A serum ferritin level may not be a useful indicator of iron-deficiency anemia in patients with what type of disorder?
    A. Chronic infection
    B. Malignancy
    C. Viral hepatitis
    D. All the above

315. Which of the following chromogens will *not* produce a colored complex with iron that can be measured spectrophotometrically?
    A. Bathophenanthroline
    B. 8-Hydroxyquinoline
    C. Tripyridyltriazine
    D. Ferrozine

316. In what disorder would an increased percent saturation of transferrin be expected?
    A. Hemochromatosis
    B. Iron-deficiency anemia
    C. Myocardial infarction
    D. Malignancy

317. Which of the following disorders is best characterized by these laboratory results?
    Serum iron—decreased
    Total iron-binding capacity—increased
    Transferrin saturation—decreased
    Serum ferritin—decreased
    Free erythrocyte protoporphyrin—increased
    A. Anemia of chronic disease
    B. Thalassemia
    C. Iron-deficiency anemia
    D. Hemochromatosis

318. Which of the following is *not* a typical finding in magnesium deficiency tetany?
    A. High serum phosphate level
    B. Normal serum calcium level
    C. Normal blood pH value
    D. Low serum potassium level

319. Which of the following constituents normally present in serum must be chemically eliminated so that it will not interfere with the measurement of serum magnesium?
    A. Calcium
    B. Chloride
    C. Iron
    D. Potassium

320. In the collection of plasma specimens for lactate determinations, which of the following anticoagulants would be more appropriate?
A. Sodium heparin
B. Sodium citrate
C. EDTA
D. Oxalate plus fluoride

321. Which of the following disorders is characterized by increased production of chloride in sweat?
A. Multiple myeloma
B. Hypoparathyroidism
C. Cystic fibrosis
D. Wilson disease

322. Which of the following is *not* a colligative property of solutions?
A. pH
B. Freezing point
C. Osmotic pressure
D. Vapor pressure

323. Which of the following describes the basis for the freezing point osmometer?
A. The freezing point depression is directly proportional to the amount of solvent present.
B. The freezing point depression varies as the logarithm of the concentration of solute.
C. The freezing point is raised by an amount that is inversely proportional to the concentration of dissolved particles in the solution.
D. The freezing point is lowered by an amount that is directly proportional to the concentration of dissolved particles in the solution.

324. Given the following information, calculate the plasma osmolality in milliosmoles per kilogram: sodium—142 mmol/L; glucose—130 mg/dL; urea nitrogen—18 mg/dL.
A. 290
B. 291
C. 295
D. 298

325. Which of the following may be associated with the colloid osmotic pressure (COP) osmometer?
A. Utilizes a cooling bath set at –7°C
B. Measures total serum osmolality
C. Negative pressure on reference (saline) side equivalent to COP of sample
D. Measures contribution of electrolytes to osmolality

Acid-Base Metabolism

326. Which is the most predominant buffer system in the body?
A. Bicarbonate/carbonic acid
B. Acetate/acetic acid
C. Phosphate/phosphorous acid
D. Hemoglobin

327. The measurement of the pressure of dissolved CO_2 (PCO_2) in the blood is most closely associated with the concentration of what substance?
A. pH
B. Bicarbonate (HCO_3^-)
C. Carbonic acid (H_2CO_3)
D. PO_2

328. What is the term that describes the sum of carbonic acid and bicarbonate in plasma?
A. Total CO_2
B. Standard bicarbonate
C. Buffer base
D. Base excess

329. To maintain a pH of 7.4 in plasma, it is necessary to maintain a
   A. 10:1 ratio of bicarbonate to carbonic acid
   B. 20:1 ratio of bicarbonate to carbonic acid
   C. 1:20 ratio of bicarbonate to carbonic acid
   D. 20:1 ratio of carbonic acid to bicarbonate

330. In the plasma, an excess in the concentration of bicarbonate without a change in PCO_2 from normal will result in what physiological state?
   A. Respiratory acidosis
   B. Respiratory alkalosis
   C. Metabolic acidosis
   D. Metabolic alkalosis

331. Which of the following characterizes respiratory acidosis?
   A. Excess of bicarbonate
   B. Deficit of bicarbonate
   C. Excess of dissolved carbon dioxide (PCO_2)
   D. Deficit of dissolved carbon dioxide (PCO_2)

332. What is the specimen of choice for analysis of acid-base disturbances involving pulmonary dysfunction in an adult?
   A. Venous blood
   B. Arterial blood
   C. Capillary blood
   D. Urine

333. What is the anticoagulant of choice for blood gas analysis?
   A. EDTA
   B. Heparin
   C. Sodium fluoride
   D. Citrate

334. If a blood gas specimen is left exposed to air, which of the following changes will occur?
   A. PO_2 and pH increase; PCO_2 decreases
   B. PO_2 and pH decrease; PCO_2 increases
   C. PO_2 increases; pH and PCO_2 decrease
   D. PO_2 decreases; pH and PCO_2 increase

335. How would blood gas parameters change if a sealed specimen is left at room temperature for 2 or more hours?
   A. PO_2 increases, PCO_2 increases, pH increases
   B. PO_2 decreases, PCO_2 decreases, pH decreases
   C. PO_2 decreases, PCO_2 increases, pH decreases
   D. PO_2 increases, PCO_2 increases, pH decreases

336. The bicarbonate ion concentration may be calculated from the total CO_2 and PCO_2 blood levels by using which of the following formulas?
   A. $0.03 \times (PCO_2 - \text{total } CO_2)$
   B. $(\text{total } CO_2 + 0.03) \times PCO_2$
   C. $0.03 \times (\text{total } CO_2 - PO_2)$
   D. $\text{total } CO_2 - (0.03 \times PCO_2)$

337. In order to maintain electrical neutrality in the red blood cell, bicarbonate leaves the red blood cell and enters the plasma through an exchange mechanism with what electrolyte?
   A. Sodium
   B. Potassium
   C. Chloride
   D. Phosphate

338. In acute diabetic ketoacidosis, which of the following laboratory findings would be expected?
   A. Fasting blood glucose elevated, pH elevated, ketone bodies present
   B. Fasting blood glucose elevated, pH low, ketone bodies present
   C. Fasting blood glucose elevated, pH normal, ketone bodies absent
   D. Fasting blood glucose decreased, pH low, ketone bodies absent

339. Which of the following is a cause of metabolic alkalosis?
   A. Late stage of salicylate poisoning
   B. Uncontrolled diabetes mellitus
   C. Renal failure
   D. Excessive vomiting

340. Which of the following statements is *true* about partially compensated respiratory alkalosis?
   A. PCO_2 is higher than normal.
   B. HCO_3^- is higher than normal.
   C. More CO_2 is eliminated through the lungs by hyperventilation.
   D. Renal reabsorption of HCO_3^- is decreased.

341. Which is a compensatory mechanism in respiratory acidosis?
   A. Hypoventilation
   B. Decreased reabsorption of bicarbonate by the kidneys
   C. Increased Na^+/H^+ exchange by the kidneys
   D. Decreased ammonia formation by the kidneys

342. Which of the following will cause a shift of the oxygen dissociation curve to the right, resulting in a decreased affinity of hemoglobin for O_2?
   A. Low plasma pH level
   B. Low PCO_2 level
   C. Low concentration of 2,3-bisphosphoglycerate
   D. Low temperature

343. Which of the following statements about carbonic anhydrase (CA) is *true?*
   A. Catalyzes conversion of CO_2 and H_2O to $HHCO_3$ in red blood cells
   B. Causes shift to the left in oxygen dissociation curve
   C. Catalyzes formation of H_2CO_3 from CO_2 and H_2O in the tissues
   D. Inactive in renal tubular cells

344. Which of the following statements best describes "base excess"?
   A. Primarily refers to carbonic acid concentration
   B. Positive values reflect metabolic alkalosis.
   C. Created through metabolism of carbohydrates
   D. Negative values represent a respiratory imbalance.

345. Given the following information, calculate the blood pH.
   PCO_2 = 44 mm Hg
   Total CO_2 = 29 mmol/L
   A. 6.28
   B. 6.76
   C. 7.42
   D. 7.44

346. A 75-year-old woman comes to her physician complaining of abdominal pain. She says she has had a sore stomach for the last 3 weeks and has been taking increasing doses of antacid pills to control it. Now she is taking a box of pills a day. Blood gases are drawn with the following results: pH = 7.49, PCO_2 = 59 mm Hg, HCO_3^- = 25 mmol/L. What do these data indicate?
   A. Metabolic alkalosis, partially compensated
   B. Respiratory acidosis, uncompensated
   C. A dual problem of acidosis
   D. An error in one of the blood gas measurements

347. A 24-year-old drug abuser is brought into the emergency department unconscious. He has shallow breaths, looks pale, and is "clammy." Blood gases show the following results: pH = 7.29, PCO_2 = 50 mm Hg, HCO_3^- = 25 mmol/L. What condition is indicated by these results?
   A. Metabolic alkalosis, partially compensated
   B. Respiratory acidosis, uncompensated
   C. A dual problem of acidosis
   D. An error in one of the blood gas measurements

348. Blood gases are drawn on a 68-year-old asthmatic who was recently admitted for treatment of a kidney infection. Blood gas results are as follows: pH = 7.25, PCO_2 = 56 mm Hg, HCO_3^- = 16 mmol/L. What condition is indicated by these results?
   A. Metabolic alkalosis, partially compensated
   B. Respiratory acidosis, uncompensated
   C. A dual problem of acidosis
   D. An error in one of the blood gas measurements

349. A mother brings her daughter, a 22-year-old medical technology student, to her physician. The patient is hyperventilating and has glossy eyes. The mother explains that her daughter is scheduled to take her final course exam the next morning. The patient had been running around frantically all day in a worried state and then started to breathe heavily. Blood gases are drawn in the office with the following results: pH = 7.58, PCO_2 = 55 mm Hg, HCO_3^- = 18 mmol/L. What do these data indicate?
   A. Metabolic alkalosis, partially compensated
   B. Respiratory acidosis, uncompensated
   C. A dual problem of acidosis
   D. An error in one of the blood gas measurements

Endocrinology

350. Secretion of hormones by the anterior pituitary may be controlled by the circulating levels of hormones from the respective target gland, as well as hormones secreted by what organ?
   A. Posterior lobe of the pituitary gland
   B. Intermediate lobe of the pituitary gland
   C. Hypothalamus
   D. Adrenal medulla

351. An elevated level of which of the following hormones will inhibit pituitary secretion of adrenocorticotropic hormone (ACTH)?
   A. Aldosterone
   B. Cortisol
   C. 17β-Estradiol
   D. Progesterone

352. Which of the following is the major mineralocorticoid?
   A. Aldosterone
   B. Cortisol
   C. Corticosterone
   D. Testosterone

353. Plasma renin activity (PRA) measurements are usually made by measuring which of the following using immunoassay?
A. Angiotensinogen
B. Angiotensin I
C. Angiotensin II
D. Angiotensin-converting enzyme

354. What effect would a low-salt diet, upright position, and diuretics have on the following test results?
A. Renin ↑, aldosterone ↑, hypernatremia, hypokalemia
B. Renin ↑, aldosterone ↓, hypernatremia, hypokalemia
C. Renin ↓, aldosterone ↓, hyponatremia, hyperkalemia
D. Renin ↓, aldosterone ↑, hyponatremia, hyperkalemia

355. As a screening test for Cushing syndrome, the physician wishes to see whether a patient exhibits normal diurnal rhythm in his or her cortisol secretion. At what time should the specimens be drawn for plasma cortisol determination?
A. 6 A.M., 2 P.M.
B. 8 A.M., 4 P.M.
C. 12 noon, 6 P.M.
D. 12 noon, 12 midnight

356. A patient is suspected of having Addison disease. His symptoms are weakness, fatigue, loss of weight, skin pigmentation, and hypoglycemia. His laboratory tests show low serum sodium and chloride, elevated serum potassium, and elevated urine sodium and chloride levels. The serum cortisol level is decreased and the plasma ACTH is increased. To make a definitive diagnosis, the physician orders an ACTH stimulation test, and serum cortisol levels are measured.

If the patient has primary hypoadrenocortical function (Addison disease), what would be the expected level of serum cortisol following stimulation? If the patient has hypopituitarism and secondary hypoadrenocortical function, what would be the expected level of serum cortisol following stimulation?
A. Increase from baseline; decrease from baseline
B. Decrease from baseline; increase from baseline
C. Slight increase from baseline; no change from baseline
D. No change from baseline; slight increase from baseline

357. What does the concentration of urinary free cortisol mainly reflect?
A. Total serum cortisol
B. Conjugated cortisol
C. Unbound serum cortisol
D. Protein-bound serum cortisol

358. A 30-year-old woman is admitted to the hospital. She has truncal obesity, buffalo humpback, moon face, purple striae, hypertension, hyperglycemia, increased facial hair, acne, and amenorrhea. The physician orders endocrine testing. The results are as follows:

Urine free cortisol—increased
Serum cortisol (8 A.M.)—increased
Plasma ACTH—decreased
Dexamethasone suppression test:
Overnight low dose—no suppression of serum cortisol
High dose—no suppression of serum cortisol

What is the most probable diagnosis?
A. Pituitary adenoma
B. Ectopic ACTH lung cancer
C. Adrenocortical carcinoma
D. Addison disease

359. Which of the following is the most common cause of the adrenogenital syndrome called congenital adrenal hyperplasia, and which test is used for its diagnosis?
   A. 17α-Hydroxylase deficiency; progesterone assay
   B. 21-Hydroxylase deficiency; 17α-hydroxyprogesterone assay
   C. 3β-Hydroxysteroid dehydrogenaseisomerase deficiency; 17α-hydroxypregnenolone assay
   D. 11β-Hydroxylase deficiency; 11-deoxycortisol assay

360. Which of the following is the most potent androgen?
   A. Androstenedione
   B. Dehydroepiandrosterone
   C. Androsterone
   D. Testosterone

361. Which of the following tissues does *not* secrete steroid hormones?
   A. Ovaries
   B. Pituitary gland
   C. Testes
   D. Adrenal cortex

362. Which of the following is the most potent estrogen and is considered to be the true ovarian hormone?
   A. Estriol (E_3)
   B. Estrone (E_1)
   C. 17β-Estradiol (E_2)
   D. 16α-Hydroxyestrone

363. During pregnancy in the second trimester, human chorionic gonadotropin (hCG) levels _____ and progesterone and estriol levels _____.
   A. Increase, increase
   B. Increase, decrease
   C. Decrease, increase
   D. Decrease, decrease

364. Which of the following is *not* quantified in the triple test for Down syndrome?
   A. α_1-Fetoprotein
   B. Unconjugated estriol
   C. Progesterone
   D. Human chorionic gonadotropin

365. Because of infertility problems, a physician would like to determine when a woman ovulates. The physician orders serial assays of plasma progesterone. From these assays, how can the physician recognize when ovulation occurs?
   A. After ovulation, progesterone rapidly increases.
   B. After ovulation, progesterone rapidly decreases.
   C. Right before ovulation, progesterone rapidly increases.
   D. There is a gradual, steady increase in progesterone throughout the menstrual cycle.

366. The placenta secretes numerous hormones both protein and steroid. Which of the following hormones is not secreted by the placenta?
   A. Human chorionic gonadotropin (hCG)
   B. Estrogen
   C. Human placental lactogen (HPL)
   D. Luteinizing hormone (LH)

367. During pregnancy, estriol is synthesized in the placenta from _____ formed in the _____.
   A. Estradiol, mother
   B. Estradiol, fetus
   C. 16α-Hydroxy-DHEA-S, mother
   D. 16α-Hydroxy-DHEA-S, fetus

368. What percentage decrease in plasma or urinary estriol, in comparison with the previous day's level, is considered significant during pregnancy?
A. 5
B. 10
C. 25
D. 40

369. Which of the following compounds is *not* a precursor of the estrogens?
A. Progesterone
B. Testosterone
C. Cholesterol
D. Aldosterone

370. When do the highest levels of gonadotropins occur?
A. During the follicular phase of the menstrual cycle
B. During the luteal phase of the menstrual cycle
C. At the midpoint of the menstrual cycle
D. Several days prior to ovulation

371. What would be an example of ectopic hormone production?
A. Prolactin production by pituitary tumors
B. Calcitonin production by thyroid tumors
C. Growth hormone production by lung tumors
D. Cortisol production by adrenal tumors

372. Which of the following hormones initiates its response by binding to cytoplasmic receptors?
A. Estradiol
B. Epinephrine
C. Growth hormone
D. Follicle-stimulating hormone

373. The adrenal medulla secretes which of the following in the greatest quantity?
A. Metanephrine
B. Norepinephrine
C. Epinephrine
D. Dopamine

374. In a patient who is suspected of having pheochromocytoma, measurement of which of the following would be most useful?
A. Metanephrine
B. Homovanillic acid
C. 5-Hydroxyindoleacetic acid
D. Homogentisic acid

375. Diabetes insipidus is associated with depressed secretion of which of the following hormones?
A. Prolactin
B. Antidiuretic hormone
C. Growth hormone
D. Oxytocin

376. A 4-year-old female presents with a palpable abdominal mass, pallor, and petechiae. Based on family history, clinical findings, and the patient's physical examination, neuroblastoma is suspected. Which of the following does *not* support such a diagnosis?
A. Increased blood dopamine levels
B. Increased blood epinephrine levels
C. Increased urinary homovanillic acid
D. Decreased urinary vanillylmandelic acid

377. Of which of the following is 5-hydroxyindoleacetic acid (5-HIAA) the primary metabolite?
A. Epinephrine
B. Norepinephrine
C. Serotonin
D. Prolactin

378. Which of the following functions as an inhibiting factor for somatotropin release?
A. Gonadotropin-releasing hormone
B. Growth hormone-releasing hormone
C. Somatomedin
D. Somatostatin

379. Which of the following is *not* associated with growth hormone?
A. Somatotropin
B. Secreted by posterior pituitary
C. Hypersecretion results in acromegaly
D. Affects lipid, carbohydrate, and protein metabolism

380. The secretion of which of the following is controlled by growth hormone?
A. Growth hormone-releasing hormone
B. Corticotropin-releasing hormone
C. Somatomedin
D. Somatostatin

381. Which of the following would be elevated in the blood in medullary carcinoma of the thyroid?
A. Calcitonin
B. Thyroxine
C. Catecholamines
D. Secretin

382. What is the predominant form of thyroid hormone in the circulation?
A. Thyroxine
B. Triiodothyronine
C. Diiodotyrosine
D. Monoiodotyrosine

383. Once synthesized, the thyroid hormones are stored as a component of thyroglobulin in what area of the thyroid gland?
A. Epithelial cell wall of the follicle
B. Colloid in the follicle
C. Isthmus of the thyroid gland
D. Extracellular space of the thyroid gland

384. How is the majority of reverse T_3 (rT_3) made?
A. Peripheral deiodination of T_4
B. Peripheral deiodination of T_3
C. From T_3 in the thyroid gland
D. From thyroglobulin in the thyroid gland

385. Which of the following is an autoantibody that binds to TSH receptor sites on thyroid cell membranes, preventing thyroid-stimulating hormone from binding?
A. Antithyroglobulin antibodies
B. Thyroid antimicrosomal antibodies
C. Thyrotropin-receptor antibodies
D. Antithyroid peroxidase antibodies

386. In a patient with suspected primary hyperthyroidism associated with Graves disease, one would expect the following laboratory serum results: free thyroxine (FT_4) _____, thyroid hormone binding ratio (THBR) _____, and thyroid-stimulating hormone (TSH) _____.
A. Increased, decreased, increased
B. Increased, decreased, decreased
C. Increased, increased, decreased
D. Decreased, decreased, increased

387. In a patient suspected of having primary myxedema, one would expect the following serum results: free thyroxine (FT_4) _____, thyroid hormone binding ratio (THBR) _____, and thyroid-stimulating hormone (TSH) _____.
A. Decreased, increased, decreased
B. Increased, increased, decreased
C. Decreased, decreased, increased
D. Increased, decreased, increased

388. Thyroid-releasing hormone (TRH) is given to a patient. Serum thyroid-stimulating hormone (TSH) levels are taken before and after the injection, and the values are the same—low. This patient probably has which of the following disorders?
A. Primary hypothyroidism
B. Secondary hypothyroidism
C. Tertiary hypothyroidism
D. Iodine deficiency

389. The presence of a very high titer for antithyroglobulin antibodies and the detection of antithyroid peroxidase antibodies is highly suggestive of what disorder?
   A. Pernicious anemia
   B. Hashimoto thyroiditis
   C. Multinodular goiter
   D. Thyroid adenoma

390. What is the major carrier protein of the thyroid hormones in the blood?
   A. Albumin
   B. Thyroxine-binding globulin
   C. Thyroxine-binding prealbumin
   D. Thyroglobulin

391. Why are the total thyroxine (T_4) levels increased in pregnant women and women who take oral contraceptives?
   A. Inappropriate iodine metabolism
   B. Changes in tissue use
   C. Changes in concentration of thyroxine-binding globulin (TBG)
   D. Changes in thyroglobulin synthesis

392. Which of the following is the Hollander insulin test used to confirm?
   A. Hyperglycemia
   B. Vagotomy
   C. Pancreatectomy
   D. Insulinoma

393. Zollinger-Ellison syndrome is characterized by an elevated blood level of which of the following?
   A. Trypsin
   B. Pepsin
   C. Gastrin
   D. Cholecystokinin-pancreozymin

394. When performing parathyroid surgery for adenoma resection, parathyroid hormone is quantified at three points relative to the surgical procedure: baseline prior to incision, second baseline with gland exposure, and third sample at post-excision. Which of the following is *not* correct in assessing the PTH values?
   A. The second baseline value should be higher than the first baseline.
   B. The first baseline value should be the highest value of the three samples.
   C. The post-excision value should be at least 50% of or lower than the second baseline.
   D. The lack of decrease in the PTH value post-excision indicates possible multigland disease.

Therapeutic Drug Monitoring and Toxicology

395. Levels of 8–9% carboxyhemoglobin saturation of whole blood are commonly found in which of the following situations?
   A. Fatal carbon monoxide poisoning
   B. Acute carbon monoxide poisoning
   C. Nonsmoking residents of rural areas
   D. Cigarette smokers

396. Which of the following methods would yield reliable quantification of ethanol in the presence of isopropanol?
   A. Reaction with permanganate and chromotropic acid
   B. Conway diffusion followed by dichromate reaction
   C. Alcohol dehydrogenase reaction
   D. Gas-liquid chromatography

397. Which of the following tests would be particularly useful in determining isopropanol exposure?
A. Serum osmolality and urine acetone
B. Urine osmolality and serum osmolality
C. Urine acetone and urine osmolality
D. Serum sodium and serum acetone

398. When screening urine for toxic concentrations of certain substances, which of the following will *not* be identified by the Reinsch test?
A. Bismuth
B. Arsenic
C. Mercury
D. Cyanide

399. Heroin is synthesized from what drug?
A. Diazepam
B. Morphine
C. Ecgonine
D. Chlorpromazine

400. After absorption, codeine is rapidly metabolized to what compound?
A. Phencyclidine
B. Morphine
C. Methadone
D. Propoxyphene

401. THC (Δ^9-tetrahydrocannabinol) is the principal active component of what drug?
A. Benzodiazepine
B. Marijuana
C. Morphine
D. Codeine

402. Identification of the urinary metabolite benzoylecgonine would be useful in determining exposure to which of the following drugs?
A. Codeine
B. Cocaine
C. Amphetamine
D. Propoxyphene

403. Of the following specimens, which would be appropriate for determining exposure to lead?
A. EDTA plasma
B. Serum
C. Whole blood
D. Cerebrospinal fluid

404. Free erythrocyte protoporphyrin (FEP) levels are useful as a screening method for exposure to which of the following metals?
A. Zinc
B. Lead
C. Iron
D. Mercury

405. Anticoagulated whole blood is the preferred specimen in determining exposure to what compound?
A. Methanol
B. Mercury
C. Acetaminophen
D. Carbon monoxide

406. What is the approximate number of half-life periods required for a serum drug concentration to reach 97–99% of the steady state?
A. 1–3
B. 2–4
C. 5–7
D. 7–9

407. For what colorimetric determination is the Trinder reaction widely used?
A. Acetaminophen
B. Propoxyphene
C. Salicylate
D. Barbiturate

408. Acetaminophen is particularly toxic to what organ?
A. Heart
B. Kidney
C. Spleen
D. Liver

409. Which of the following is an example of a long-acting barbiturate?
A. Phenobarbital
B. Amobarbital
C. Secobarbital
D. Pentobarbital

410. Increased trough levels of aminoglycosides in the serum are often associated with toxic effects to which organ?
A. Heart
B. Kidney
C. Pancreas
D. Liver

411. Which of the following is an example of an antiarrhythmic drug that has a metabolite with the same action?
A. Quinidine
B. Digoxin
C. Procainamide
D. Nortriptyline

412. In what form must a drug be in order to elicit a pharmacologic response?
A. Free
B. Bound to albumin
C. Bound to globulins
D. Bound to fatty acids

413. An epileptic patient receiving phenytoin develops acute glomerulonephritis. What change, if any, would be expected in the patient's circulating drug level?
A. Decrease in free drug
B. Increase in free drug
C. Increase in protein-bound drug
D. No change in circulating drug level

414. Free drug levels can generally be determined by analyzing what body fluid?
A. Whole blood
B. Ultrafiltrate of plasma
C. Urine
D. Protein-free filtrate of plasma

415. Which of the following drugs is used as an immunosuppressant in organ transplantation, especially in liver transplants?
A. Methotrexate
B. Amiodarone
C. Tacrolimus
D. Paroxetine

416. Which of the following is a commonly encountered xanthine that could potentially interfere with the determination of theophylline?
A. Nicotine
B. Caffeine
C. Amphetamine
D. Procainamide

417. What is the major active metabolite of the anticonvulsant drug primidone?
A. Phenytoin
B. Acetazolamide
C. NAPA
D. Phenobarbital

418. Nortriptyline is the active metabolite of which of the following drugs?
A. Amitriptyline
B. Desipramine
C. Imipramine
D. Doxepin

419. Which of the following is used in the treatment of manic depression?
A. Potassium
B. Lithium
C. Calcium
D. Chloride

420. When is a blood sample for determination of the trough level of a drug appropriately drawn?
A. During the absorption phase of the drug
B. During the distribution phase of the drug
C. Shortly before drug administration
D. Two hours after drug administration

421. In regard to drug distribution patterns, which of the following statements is *false?*
   A. Drug metabolism is slower in newborns than adults.
   B. Drug metabolism is more rapid for 6-year-old children than for adults.
   C. Renal clearance of drugs is faster in newborns than adults.
   D. Drug metabolism often changes during pubescence.

422. Which of the following serum components is able to alter the free drug level in plasma?
   A. Creatinine
   B. Urea
   C. Albumin
   D. Calcium

423. Which of the following is an example of a phenothiazine drug?
   A. Cyclosporine
   B. Theophylline
   C. Phenytoin
   D. Chlorpromazine

424. What is the recommended name for diphenylhydantoin?
   A. Phenytoin
   B. Nalorphine
   C. Primidone
   D. Carbamazepine

425. Which of the following classes of compounds has a sedative effect and as such is used to treat anxiety?
   A. Amphetamines
   B. Opiates
   C. Cannabinoids
   D. Benzodiazepines

426. What is the active metabolite of the antiarrhythmic drug procainamide?
   A. Pronestyl
   B. Disopyramide
   C. PEMA
   D. NAPA

427. Which of the following drugs is used as a bronchodilator?
   A. Theophylline
   B. Phenytoin
   C. Amikacin
   D. Clozapine

Vitamins

428. Which of the following techniques is more commonly used to measure vitamins?
   A. High-performance liquid chromatography
   B. Spectrophotometry
   C. Nephelometry
   D. Microbiological

429. In the United States, most cases of scurvy occur in children between the ages of 7 months to 2 years. Scurvy is a disease caused by a deficiency in which of the following?
   A. Vitamin A
   B. Vitamin C
   C. Vitamin D
   D. Vitamin K

430. The term "lipid" encompasses a wide variety of compounds characterized as being insoluble in water but soluble in nonpolar solvents. Which of the following vitamins is *not* classified as fat soluble?
   A. Vitamin A
   B. Vitamin C
   C. Vitamin D
   D. Vitamin E

431. Measuring which of the following compounds is useful in the diagnosis of steatorrhea?
   A. Vitamin B_{12}
   B. Vitamin C
   C. Carotenoids
   D. Folic acid

432. Which of the following is another name for vitamin B_{12}?
A. Retinol
B. Pyridoxine
C. Cyanocobalamin
D. Riboflavin

433. Which of the following is *not* associated with vitamin B_{12}?
A. Insoluble in water
B. Intrinsic factor
C. Schilling test
D. Pernicious anemia

434. Which of the following tissues is important in vitamin D metabolism?
A. Skin
B. Spleen
C. Pancreas
D. Thyroid

435. A deficiency in which of the following leads to increased clotting time and may result in hemorrhagic disease in infancy?
A. Riboflavin
B. Pyridoxine
C. Tocopherols
D. Menaquinone

436. Which vitamin is a constituent of two redox coenzymes?
A. Vitamin A
B. Vitamin B_2
C. Vitamin B_6
D. Vitamin C

437. Which disorder is associated with thiamin deficiency?
A. Beriberi
B. Pellagra
C. Rickets
D. Dermatitis

answers & rationales

Instrumentation and Analytical Principles

1.

A. A tungsten-filament lamp is the most common light source for photometry in the visible region. It provides a continuous spectrum (360–800 nm) from the near infrared (IR) through the visible to the near ultraviolet (UV) region. Most of the radiant energy is in the near IR. Only about 15% is in the visible region—the region usually used. Because of the large emission in the near IR, tungsten lamps generate a significant amount of heat. Hydrogen and deuterium lamps are used for work in the 200–375 nm range. The mercury vapor lamp does not provide a continuous spectrum, emitting radiation at specific wavelengths.

2.

B. Photometric methods are based on the use of Beer's law, which is applicable only for monochromatic light. A monochromator is a device for selecting a narrow band of wavelengths from a continuous spectrum. The three kinds of monochromators are filters, prisms, and diffraction gratings.

3.

C. A photomultiplier tube (PMT) responds to the radiant energy (light) it absorbs by emitting electrons in a proportional amount to the initial light absorbed. These electrons then go through a series of stages where amplification occurs. The cascade effect, as the electrons go through 10 to 15 stages, results in a final current that may be one million times the initial current. The PMT exhibits rapid response time and sensitivity. These qualities also dictate that this type of detector be shielded from stray light and room light to prevent burnout. The rapid response time of a PMT makes it able to monitor interrupted light beams produced by a chopper.

4.

D. A photomultiplier tube (PMT) has two functions: (1) It is a transducer that converts light to electricity; and (2) it amplifies the signal within the tube. Amplification can be as great as one million times. The emission of electrons by a light-sensitive surface—that is, the conversion of light energy to electrical energy—is virtually instantaneous. Hence, PMTs have a very rapid response time. An iron plate and a layer of selenium are partial descriptions of the composition of a photocell or barrier layer cell.

5.

D. Photodiode array detectors are designed with 256 to 2048 photodiodes that are arranged in a linear fashion. This arrangement allows each photodiode to respond to a specific wavelength that results in a continuous UV/visible spectrum. Resolution is generally 1–2 nm.

6.

D. Wavelength calibration of a spectrophotometer is performed to verify that the radiant energy emitted from the monochromator through the exit slit is the same as the wavelength selector indicates. The glass filters holmium oxide, used in the UV and visible ranges, and didymium, used in the visible and near IR regions, are employed to check wavelength accuracy. Solutions of stable chromogens such as nickel sulfate may be used. Source lamps may be replaced with mercury-vapor or deuterium lamps. These lamps have strong emission lines and provide the most accurate method of wavelength calibration.

7.

D. The reagent blank contains the same reagents as those used for assaying the specimen. By adjusting the spectrophotometer to 100%T (or 0 absorbance) with the reagent blank, the instrument automatically subtracts the color contributed by the reagents from each succeeding reading of specimens, controls, and standards. This technique is used both in manual procedures and automated instruments. Because the reagent blank does not contain sample, there is no correction for interfering chromogens or lipemia.

8.

A. Measurement of an assay at two different wavelengths is termed bichromatic. The wavelengths chosen for absorbance readings will represent the peak and base of the spectral absorbance curve for the particular assay. By determining the difference between the two measured absorbances, the sample's concentration can be calculated with elimination of background interference from such substances as bilirubin and hemoglobin. Thus, bichromatic analysis functions as a reference blank for each individual sample.

9.

C. The bandpass or bandwidth is the range of wavelengths that are passed by a monochromator. In the example given, the bandpass will permit a 10-nm range of wavelengths to pass through the monochromator and impinge on the sample solution in the cuvet. Thus, 540 ± 5 nm (10-nm bandpass) will be equivalent to a wavelength range of 535–545 nm.

10.

A. When the absorbance of a sample in solution varies directly with the concentration of the sample, Beer's law is followed. In turn, when the absorbance increases exponentially with an increase in the light path, the Lambert law is followed. Incorporation of these two laws may be stated as $A = abc$, where A = absorbance, a = absorptivity of the substance being measured, b = light path in cm, and c = concentration of the measured substance. When the Beer-Lambert law is applied to spectrophotometric analyses of standards and unknown samples that are being measured, the following equation is derived: $A_u \times C_s/A_s = C_u$, where A_u = absorbance of unknown, C_u = concentration of unknown, A_s = absorbance of standard, and A_u = absorbance of unknown. This formula is applied to assays that exhibit linear relationships between changes in absorbance with changes in concentration to calculate the concentration of the unknown sample.

11.

C. In spectrophotometry, molecules in solution will cause incident light to be absorbed while the remaining light energy will be transmitted. Absorbance is the term used to describe the monochromatic light that is absorbed by the sample, and transmittance describes the light that passes through the sample. The mathematical relationship between absorbance and transmittance is expressed by $A = 2 - \log \%T$.

12.

D. Turbidimetry is the measurement of the amount of light blocked by particulate matter in passing through a turbid solution. The amount of light blocked depends on the number and the size of the particles. Hence the particle size in samples and standards must be comparable. Consistent timing of sample preparation and assay helps to avoid errors resulting from aggregation or settling of particles. The procedure is usually carried out at room temperature. Slight variations in temperature are not critical.

13.

C. In the dry reagent slide technique, as light from a radiant energy source passes through an interference filter, it is projected to the slide at a 45-degree angle. The light then follows a path through the clear support material and reagent layer and hits a white spreading layer; the unabsorbed light is then reflected back through the reagent and support layers. This reflected light impinges on the photodetector, which is positioned at a 90-degree angle to the slide. Because reflectance values are neither linearly proportional to transmission values nor consequently to dye concentration, the microcomputer utilizes an algorithm as a linearizing transformation of reflectance values to transmission values so that concentration may be calculated.

14.

D. In a fluorometer, light from the excitation lamp travels in a straight line, whereas the fluorescent light is radiated in all directions. If the detector for the emitted fluorescent light is placed at a right angle to the path of the excitation light, the excitation light will not fall on the detector. In addition, baffles can be placed around the cuvet to avoid reflection of the exciting light from the surface of the cuvet to the detector. The right-angle configuration does not prevent loss of the exciting or the emitted light.

15.

A. Fluorescence occurs when a molecule absorbs light of a particular wavelength and is thereby stimulated to emit light of a longer wavelength. The emitted light has a characteristic spectrum, the emission spectrum, that is unique for each fluorescing molecule. Hence, fluorometric methods are extremely sensitive and highly specific. Because of this extreme sensitivity, reagents used must be of a higher degree of purity than is required for spectroscopy, because even slight traces of impurities may fluoresce.

16.

A. Instrumentation employing fluorescence polarization is used for such testing as therapeutic drug levels and fetal lung maturity analysis. In these immunologic assays, plane-polarized light excites fluorophors in the sample cuvet. The free fluorophore-labeled ligands rotate freely because of their small size and primarily emit depolarized light. The labeled ligand-antibody complexes rotate more slowly because of their large size and emit polarized fluorescent light. Because of the differences in emitted light, it is not necessary to separate free from bound fluorophore-labeled ligands, allowing for use of the homogeneous assay technique. The emitted fluorescence intensity is measured by a polarization analyzer in the vertical plane, followed by its 90-degree movement for measurement in the horizontal plane. The amount of polarized light detected is inversely proportional to the concentration of ligand in the serum sample.

17.

A. Bioluminescence is a type of chemiluminescence in which the excitation energy is supplied by an enzymatic chemical reaction rather than by radiant energy, as in fluorescence and phosphorescence. Bioluminescence assays may employ such systems as NADH:FMN oxidoreductase-bacterial luciferase or adenosine triphosphate-firefly luciferase. Bioluminescence assays are nonradioactive, having sensitivity levels in the attomole (10^{-18}) to zeptomole (10^{-21}) ranges, which makes them more sensitive than direct fluorescence assays. Bioluminescence has been applied in the development of immunoassays.

18.

B. Nephelometry is the measurement of the amount of light scattered by particles in suspension. The amount of light scattered depends on the size and shape of the particles and on the wavelength of the incident light. Ultraviolet light should not be used because it might produce some fluorescence, which would lead to erroneously high results.

19.

C. Radionuclides are quantified by measuring the amount of energy that they emit. This can be in the form of alpha emission ${}_2^4He^{2+}$, beta emission (electrons ejected from the nucleus of a radioisotope during radioactive decay), or gamma emission (electromagnetic radiation emitted during radioactive decay). Beta and gamma emissions can be detected by scintillation counters. The sensing element of a scintillation counter is a fluor, a substance capable of converting radiation energy to light energy. The light energy is converted to electrical energy and amplified by a photomultiplier tube. A fluor commonly employed in solid scintillation counters is a large crystal of sodium iodide containing a small amount of thallium as an activator; it is used for gamma counting. Beta emission is counted by liquid scintillation counters using fluors dissolved in organic solvents. Alpha emission has very low penetrating power and is not measured in the clinical laboratory. Although radioimmunoassay (RIA) is no longer used for routine analyses and has been replaced by nonradioactive immunoassays, it is still used in a limited manner in some clinical reference laboratories and in research settings.

20.

C. Chemiluminescence is a type of luminescence where excitation does not require absorption of radiant energy. Chemiluminescence is the process where the chemical energy of a reaction produces excited atoms, and upon electron return to ground state photons of light are emitted. Chemiluminescence has been applied in the development of immunoassays and has ultrasensitivity in the attomole (10^{-18}) to zeptomole (10^{-21}) ranges.

21.

D. Atomic absorption spectrophotometry (AAS) is based on the principle that atoms in a basic ground state are capable of absorbing energy in the form of light at a specific wavelength. In a single-beam AAS, the amount of light that the analyte absorbs from the hollow-cathode lamp is what we wish to know. However, what is actually measured is the intensity of the beam after it has passed through the flame. This measurement is made with and without sample in the flame. In this way, the instrument calculates the amount of light absorbed because of the presence of the analyte in the flame. Because most samples usually have the analyte in the form of a compound or an ion, the analyte must first be converted to nonionized atoms. This is achieved by heating in a flame. About 99% of the atoms of analyte in the flame are in the ground state and, therefore, are capable of absorbing energy at the appropriate wavelength. Hence, light absorbed is essentially proportional to the concentration of the analyte. The light source in AAS is a hollow-cathode lamp in which the cathode contains the element that is to be measured.

22.

C. The basis of AAS is the measurement of light, at a specific wavelength, that is absorbed by an element whose atoms are in a ground state. The flame in AAS serves two functions—to accept the sample, thus serving as a cuvet, and to supply heat for converting the element, which is usually present in the sample in molecular form, into its atomic form at ground-state energy level. The hollow-cathode lamp supplies the emission line of light required for the analysis. The metal element of interest is coated on the cathode of the lamp. When the inert gas, either argon or neon, becomes ionized, it is drawn toward the cathode. The impact excites the metal element coated on the cathode, resulting in the emission of spectral lines specific for the element. This light emission is then absorbed by the metal element in the sample. A flameless AAS employs a carbon rod (graphite furnace), tantalum, or platinum to hold the sample in a chamber. The temperature is raised to vaporize the sample being analyzed. The atomized sample then absorbs the light energy from the hollow-cathode lamp. This technique is more sensitive than the flame method.

23.

D. A beam chopper is a device for interrupting a beam of light so that a pulsed beam is produced. In an atomic absorption spectrophotometer, if the light entering the flame from the hollow-cathode lamp is pulsed, then the light leaving the flame will consist of unabsorbed pulsed light and unpulsed light from the flame and from a small amount of emission by excited atoms of the analyte. The detector has an amplifier that is tuned to recognize and amplify only the pulsed signal. Thus errors caused by light from the flame and light emitted by the analyte are avoided. However, the beam chopper and tuned amplifier do not compensate for errors introduced by variations in flame temperature or deterioration of the hollow-cathode lamp. AAS may be used to measure such analytes as lead, zinc, copper, aluminum, magnesium, calcium, and lithium.

24.

A. A half-cell, also called an electrode, is composed of a single metallic conductor surrounded by a solution of electrolyte. An electrochemical cell consists of two half-cells. If two different kinds of half-cells are connected in such a way as to make a complete circuit, a current will flow because of the potential difference between the two electrodes. The connection must be between the two metallic conductors and also between the two electrolyte solutions, usually by means of a salt bridge. In the analytical technique of potentiometry, a comparison is made between the voltage of one half-cell connected to another half-cell. It is customary that all half-cell potentials be compared to the potential generated by a standard electrode. The universally accepted standard half-cell with which all other half-cells are compared is the standard hydrogen electrode, arbitrarily assigned a potential E° of 0.000 volt.

25.

B. Oxidation involves the loss of electrons, and reduction the gain of electrons. In an electrolytic cell composed of two different half-cells—for example, zinc in zinc sulfate and copper in copper sulfate—electrons will flow from the anode to the cathode. Thus reduction takes place at the cathode, whereas oxidation occurs at the anode. "Combination electrode" refers to the combining of indicator and reference electrodes into a single unit. "Electrode response" refers to the ability of an ion-selective electrode to respond to a change in concentration of the ion being measured by exhibiting a change in potential.

26.

B. In practical applications of potentiometry, it is desirable to use one half-cell with a known and constant potential that is not sensitive to the composition of the material to be analyzed. This is called the reference electrode. One type of reference electrode is the calomel electrode, which consists of mercury covered by a layer of mercurous chloride in contact with a saturated solution of potassium chloride. The other half-cell, called the indicator electrode, is selected on the basis of the change in its potential with change in the concentration of the analyte of interest. The silver-silver chloride electrode is a commonly used type of reference electrode. The sodium and calcium electrodes are types of ion-selective electrodes.

27.

C. For optimum performance, pH-sensitive glass electrodes that are not actively in use should be kept immersed in an aqueous medium. Because the exact composition of the pH-sensitive glass varies from one manufacturer to another, the glass electrode should be maintained in the medium recommended by the manufacturer. Usual media are deionized water, dilute HCl, and buffer with a pH near the pH of the solution to be measured. The functioning of a glass electrode depends on the properties of the pH-sensitive glass. A typical glass electrode is made by sealing a thin piece of pH-sensitive glass at the end of a piece of glass tubing and filling the tube with a solution of hydrochloric acid saturated with silver chloride. A silver wire is immersed in the solution in the tube, with one end extending outside the tube for external connection. This is essentially a silver/silver chloride reference electrode sealed within the tube with the pH-sensitive glass tip. This pH-sensitive glass functions appropriately only when it is saturated with water. Then each surface of the glass develops a hydrated lattice, where exchange of alkaline metal ions in the lattice for hydrogen ions in the test solution can occur.

28.

D. The ion-exchange electrode is a type of potentiometric, ion-selective electrode that consists of a liquid ion-exchange membrane that is made of an inert solvent and an ion-selective neutral carrier material. A collodion membrane may be used to separate the membrane solution from the sample solution being analyzed. Because of its ability to bind K^+, the antibiotic valinomycin is used as the neutral carrier for the K^+-selective membrane. The antibiotics nonactin and monactin are used in combination as the neutral carrier for the NH_4^+-selective membrane. A special formulation is used to make a selective glass membrane for the measurement of sodium.

29.

C. Ion-selective electrodes for the measurement of sodium are glass membrane electrodes with selective capability. They are constructed from glass that consists of silicon dioxide, sodium oxide, and aluminum oxide. This type of electrode is based on the principle of potentiometry. Measurement errors may occur from protein buildup on the membrane surface. Potassium is measured using an ion-exchange electrode where the liquid ion-exchange membrane consists of valinomycin as the ion-selective carrier.

30.

D. A chloride coulometer employs a coulometric system based on Faraday's law, which states that in an electrochemical system, the number of equivalent weights of a reactant oxidized or reduced is directly proportional to the quantity of electricity used in the reaction. The quantity of electricity is measured in coulombs. The coulomb is the unit of electrical quantity; 1 coulomb of electricity flowing per minute constitutes a current of 1 ampere. Thus, if the current is constant, the number of equivalent weights of reactant oxidized or reduced depends only on the duration of the current. In the chloride coulometer, the electrochemical reaction is the generation of Ag^+ ions by the passage of a direct current across a pair of silver electrodes immersed in a conducting solution containing the sample to be assayed for chloride. As the Ag^+ ions are generated, they are immediately removed from solution by combining with chloride to form insoluble silver chloride. When all the chloride is precipitated, further generation of Ag^+ ions causes an increase in conductivity of the solution. Thus the instrument provides an electrometric titration, in which the titrant is Ag^+ ions and the endpoint of the titration is indicated by the increase in conductivity of the solution. Amperometry is used to measure the increase in conductivity. The amperometric circuit includes a second pair of silver electrodes that are immersed in the solution. They are provided with a small, steady, and constant voltage. The appearance of free Ag^+ ions in the solution generates a sharp increase in conductivity, which, in turn, causes a sudden rise in the current between the electrodes in the amperometric circuit. This increase in current activates a relay that stops the further generation of Ag^+ ions and also stops an automatic timer placed in the circuit to measure the total duration of current in the coulometric circuit. Although this system is no longer used for routine analysis of serum, it is still employed for sweat chloride analysis.

31.

C. In an amperometric glucose electrode system, glucose oxidase reacts with glucose to produce hydrogen peroxide and gluconic acid. The platinum electrode that operates at a positive potential oxidizes the hydrogen peroxide to oxygen. The oxidation of hydrogen peroxide produces a current that is directly proportional to the glucose level in the sample.

32.

D. A pH/blood gas analyzer contains a pH-sensitive glass electrode, a PCO_2 electrode, and a PO_2 electrode. The glass electrode is calibrated by comparison with two primary standard buffers of known pH. Because pH readings are temperature sensitive, the calibration must be carried out at a constant temperature of 37°C. pH readings are not appreciably sensitive to changes in barometric pressure. Note that if the PCO_2 and PO_2 electrodes were also to be calibrated, then it would be essential to know the barometric pressure, because that affects the PCO_2 and PO_2 calibrating gases.

33.

B. In a blood gas analyzer, the PCO_2 electrode is actually a pH electrode immersed in a bicarbonate solution. The bicarbonate solution is separated from the sample by a membrane that is permeable to gaseous CO_2 but not to ionized substances such as H^+ ions. When CO_2 from the sample diffuses across the membrane, it dissolves, forming carbonic acid and thus lowering the pH. The pH is inversely proportional to the log of the PCO_2. Hence the scale of the meter can be calibrated directly in terms of PCO_2. It should be noted that whereas pH refers to the negative logarithm of the H^+ ion concentration, PCO_2 refers to the partial pressure of CO_2.

34.

B. In a blood gas analyzer, the electrode for measuring the partial pressure of oxygen (PO_2) in the blood is an electrochemical cell consisting of a platinum cathode and a Ag/AgCl anode connected to an external voltage source. The cathode and anode are immersed in buffer. A polypropylene membrane selectively permeable to gases separates the buffer from the blood sample. When there is no oxygen diffusing into the buffer, there is practically no current flowing between the cathode and the anode because they are polarized. When oxygen diffuses into the buffer from a sample, it is reduced at the cathode. The electrons necessary for this reduction are produced at the anode. Hence a current flows; the current is directly proportional to the PO_2 in the sample.

35.

C. pH, PCO_2, and PO_2 are measured directly from the specimen by utilizing electrodes. The pH and PCO_2 electrodes are potentiometric where the voltage produced across a semipermeable membrane to hydrogen ions or CO_2 gas is proportional to the "activity" of those ions in the patient's sample. Activity is measured in voltage whose value can be presented in terms of concentration. PO_2 is measured similarly, but using an amperometric electrode. For PO_2 a small charge is put on a cathode, and electrons are drawn off the cathode in proportion to the oxygen present. The O_2 becomes part of the circuit. The amount of electrons drawn is proportional to the amount of oxygen present. Bicarbonate and other parameters, such as base excess, are calculated by the instrument using pH and PCO_2 values and the Henderson/Hasselbalch equation.

36.

A. In polarography, an electrochemical cell is used. A gradually increasing voltage is applied between the two electrodes of the cell that are in contact with a solution containing the analyte. The current flowing in the system is measured. Plotting the voltage change versus current change gives a polarogram. The voltage at which the sharp rise in current occurs is characteristic of the electrochemical reaction involved; that is, characteristic of the analyte. The amount of increase in current (i.e., the wave height) is proportional to the concentration of analyte. In anodic stripping voltammetry, a negative potential is applied to one of the electrodes. Trace metal ions in the solution are thereby reduced and plated onto the anodic electrode. This is a preconcentrating step. The plated electrode is then used as the anode in a polarographic cell. The metal is thereby stripped off the anode. The current flow during the stripping provides a polarogram that both identifies and quantifies the trace metals. The method is particularly appropriate for assaying heavy metals such as lead in blood.

37.

B. Electrophoresis is a method of separating charged particles by their rates of migration in an electric field. An electrophoretic chamber consists of two electrodes, two reservoirs to hold buffer, a means of supporting a strip in the chamber so that the ends are dipping into the reservoirs, and a means of applying an electric current to the strip. The whole chamber is sealed to make it vaporproof.

38.

A. Capillary electrophoresis is based on electroosmotic flow (EOF). When an electric field is applied, the flow of liquid is in the direction of the cathode. Thus, EOF regulates the speed at which solutes move through the capillary. Cations migrate the fastest, because EOF and electrophoretic attraction are in the direction of the cathode.

39.

D. When serum is applied to a support medium placed in a buffer solution of alkaline pH and subjected to an electrical field, the serum proteins will be separated into fractions for identification and quantification. Support media that may be used for electrophoretic separations include agarose gel, starch gel, cellulose acetate, and acrylamide. The pore size of the agarose gel and cellulose acetate is large enough that the protein molecules are able to move freely through the media with a resolution of between five to seven fractions. Because the pore size of starch gel and acrylamide is somewhat smaller, the resolution of approximately 20 fractions is possible with this type of medium. Agarose gel and cellulose acetate are the more commonly used media in the routine clinical laboratory. Celite provides the inert supporting phase in gas-liquid chromatography.

40.

A. Buffer solutions of pH 8.6 are commonly used for serum protein electrophoresis. At this alkaline pH, the serum proteins have a net negative charge. Therefore, the negatively charged serum proteins migrate toward the anode. This is true for all the proteins except the gamma-globulins, which tend to show the phenomenon of endosmosis.

41.

C. Proteins are dipolar or zwitterion compounds because they contain amino acids that exhibit both negative and positive charges. The isoelectric point (pI) of a protein refers to the pH at which the number of positive charges on the protein molecule equals the number of negative charges, causing the protein to have a net charge of zero. Because the protein exhibits electrical neutrality at its isoelectric point, it is unable to migrate in an electrical field.

42.

C. Amido black 10B, Coomassie brilliant blue, and Ponceau S are dyes that are used to stain serum proteins after electrophoresis. Once the serum protein bands are stained, they may be quantified by scanning the support media at the appropriate wavelength with a densitometer. Oil red O and fat red 7B are dyes that are used to stain lipoproteins following electrophoresis.

43.

D. In electrophoresis, each band in the stained protein pattern should be uniformly colored; that is, no holes should appear within an individual band. Such a doughnut-like appearance occurs when the protein is present in too high a concentration, thus exceeding the complexing ability of the stain. To overcome this problem, dilute elevated specimens before rerunning the electrophoresis.

44.

D. Ampholytes are mixtures of polyanions and polycations used to establish a pH gradient within the gel media in isoelectric focusing. When an electrical field is applied to the gel, ampholytes seek their own isoelectric point where they become stationary, establishing a pH gradient. Similarly, proteins will migrate within the gel-gradient until they reach the pH of their isoelectric point, thus becoming stationary or focused. This system is most useful in separating proteins that have close isoelectric points.

45.

C. Silver stains react with nanogram concentrations of proteins and nucleic acids, staining them shades of green, yellow, blue, and red. Silver stains are approximately 30 times more sensitive than Coomassie blue stains. Because of their sensitivity, silver stains are being used in electrophoretic methods to identify cerebrospinal fluid and urine proteins without preconcentration of the specimens.

46.

B. Isoelectric focusing is a type of zone electrophoresis. It requires the establishment of a pH gradient, within the agarose or polyacrylamide gel medium, to obtain the separation of charged proteins. Under constant power, the proteins migrate to the pH that corresponds to the isoelectric point of the particular protein.

47.

C. Protein molecules can exist as anions, cations, or zwitterions, depending on the pH of the solution in which they are placed. The pH at which they exist in the form of zwitterions and hence have no net charge is called the isoelectric point. The principle of isoelectric focusing is based on the ability to separate proteins because of differences in their isoelectric points. Aliphatic polyamino polycarboxylic acids, known as ampholytes, are used to produce the pH gradient.

48.

B. In thin-layer chromatography (TLC), the R_f (retention factor) describes the distance traveled by the solute (compound of interest) in relation to the distance traveled by the solvent (mobile phase). Measurements of the TLC plate are made from the origin or point of sample application to the center of the developed spot and from the origin to the solvent front. An R_f may be calculated by means of the following formula:

$$R_f = \frac{\text{Distance from origin to spot center}}{\text{Distance from origin to solvent front}}$$

$$R_f = \frac{48 \text{ mm}}{141 \text{ mm}} = 0.34$$

The R_f of the compound of interest, along with chromogenic spray characteristics, may then be compared with standards for identification of the unknown compound.

49.

C. The column and carrier gas flow rate used in gas-liquid chromatography are important aspects of the separation and resolving power of the system. When the column eluent is introduced into a mass spectrometer, additional information pertaining to elemental composition, position of functional groups, and molecular weight may be determined for the purpose of identifying compounds (e.g., drugs in biological samples). Mass spectrometers consist of a vacuum system, ion source, mass filter, and detector.

50.

C. High-performance liquid chromatography (HPLC) systems are composed of four basic units: sample-introduction system, solvent-delivery system, column, and detector. The sample-introduction system is generally a fixed-loop injection valve, which allows the sample to be injected into a stainless steel external loop for flushing onto the column by the solvent. The solvent-delivery system may be composed of one or two pumps for the purpose of forcing the mobile phase and sample through the column. Photometric, fluorometric, and electrochemical detectors are available for monitoring the eluate as it emerges from the column.

51.

C. In HPLC, the technique used for the mobile phase may be isocratic or gradient elution. With isocratic elution the strength of the solvent remains constant during the separation. With gradient elution the strength of the solvent is continually increased (percent per minute) during the separation process. The gradient elution technique is sometimes employed to improve HPLC resolution and sensitivity.

52.

A. Discrete analyzers are designed so that each specimen-reagent mixture is analyzed separately in its own vessel. Although a discrete analyzer may be designed to measure only one analyte, most discrete analyzers are very versatile and are able to run multiple tests on each sample. Some discrete analyzers also have random access capability that allows STAT samples to be accessed easily.

53.

B. High-performance liquid chromatography is also called high-pressure liquid chromatography. It is a form of column chromatography in which a liquid moving phase is actively pumped through the column, thus speeding the separation process considerably. HPLC is used in therapeutic drug monitoring and in assaying vitamin and hormone concentrations.

54.

A. Chromatography provides a variety of means of separating mixtures of substances on the basis of their physicochemical properties, primarily their solubility in a variety of solvents. Chromatographic methods always involve a stationary phase and a mobile phase. The sample containing the substances to be separated is carried in the mobile phase; the mobile phase passes over the stationary phase at different rates depending on their relative solubilities in the two phases. The amount of separation depends on (1) the rate of diffusion, (2) the solubility of the substances being separated, and (3) the nature of the solvent. In TLC, the stationary phase is a thin layer of some sorbent such as silica gel uniformly spread on a piece of glass or plastic.

55.

D. In gas-liquid chromatography (GLC), the stationary phase is a liquid adsorbed on particles packed in a column. The mobile phase is a gas that passes through the column. Because the sample is carried in the mobile phase, it must be volatile at the temperature of the column so that it can be carried by the gas. In addition, separation is dependent on the solubility of the solute in the liquid layer of the stationary phase.

56.

B. Ion-exchange chromatography uses synthetic ion-exchange resins. They may be cation- or anion-exchange resins. They can be used in either a column or a thin layer. Separation of mixtures of substances by ion-exchange chromatography depends primarily on the sign and the ionic charge density of the substances being separated.

57.

A. Mass spectrometry identifies a compound based on the principle of charged particles moving through a magnetic or electric field, with ions being separated from other charged particles according to their mass-to-charge ratios. The mass spectrum produced is unique for a particular compound. It also identifies the positioning of functional groups of the compound. Mass spectrometry is useful in the clinical laboratory for drug identification.

58.

D. Mass spectrometry is used in the clinical laboratory in conjunction with gas or liquid chromatography (GC-MS). In gas chromatography a compound is identified by its retention time. If two compounds have very similar retention times, the compound may be misidentified. Gas chromatography complements mass spectrometry in that the eluted peak is subjected to mass spectrometric analysis for molecular weight determination. Use of the two systems in tandem allows for more accurate identification of compounds.

59.

D. With automated instruments, the quality of the specimen and its handling are critical to producing accurate test results. Sampling errors can occur that cause falsely low results to be generated. These errors include short sampling, air pocket in the bottom of the sample cup, and fibrin clots in the sample probe.

60.

C. As part of a good quality assurance program, a laboratory should perform function verification, performance verification, and preventive maintenance for all instrument systems. Function verification is the monitoring of specific instrument functions and the correcting of these functions when necessary to assure reliable operation. Function verification includes monitoring temperature, setting electronic parameters, calibrating instruments, and analyzing quality control data. It is important that performance of these activities be properly documented.

61.

D. It is imperative that preventive maintenance procedures be performed and the results recorded for all laboratory instrumentation. This includes maintenance of analytical balances, refrigerators, freezers, centrifuges, ovens, water baths, heating blocks, thermometers, pipetters, dilutors, automated analyzers, and all other laboratory equipment used for analyzing specimens. Preventive maintenance is performed at scheduled times such as per shift, daily, weekly, monthly, or yearly.

62.

D. In order to prevent excessive downtime and costly repairs, a preventive maintenance schedule should be devised, implemented, and recorded for all laboratory equipment. Preventive maintenance procedures include the cleaning of instrument components, the replacing of worn parts, and the adjusting of certain parts or parameters. Following a preventive maintenance schedule will help to extend the life of the equipment. It is important that all laboratory personnel recognize the need for routine maintenance and follow prescribed maintenance schedules.

63.

A. Hemoglobin is a tetramer composed of four globin chains, four heme groups, and four iron atoms. In adult hemoglobin, or hemoglobin A_1, there are two alpha chains and two beta chains. Hemoglobin A_2, which comprises less than 4% of the normal adult hemoglobin, is composed of two alpha chains and two delta chains. Hemoglobin F, or fetal hemoglobin, is composed of two alpha chains and two gamma chains.

64.

D. Although hemoglobin differentiation is best achieved by use of electrophoresis, hemoglobin F may be differentiated from the majority of human hemoglobins because of its alkali resistance. Hemoglobin F is able to resist denaturation and remain soluble when added to an alkaline solution. In contrast to hemoglobin F, most hemoglobins will denature in alkaline solution and precipitate on the addition of ammonium sulfate. After 1 year of age, the normal concentration of hemoglobin F is less than 1% of the total hemoglobin. However, hemoglobin F may be present in elevated concentrations in disorders that include thalassemia, sickle cell disease, and aplastic anemia.

65.

A. A number of hemoglobinopathies exist where a substitution of one amino acid on either the alpha chain or the beta chain causes the formation of an abnormal hemoglobin molecule. Hemoglobin S is an abnormal hemoglobin that is characterized by the substitution of valine for glutamic acid in position 6 of the beta chain. Hemoglobin C is an abnormal hemoglobin in which lysine replaces glutamic acid in position 6 of the beta chain. The structural changes that are seen in hemoglobins S and C disorders are inherited as autosomal recessive traits.

66.

A. At pH 8.6, hemoglobins have a net negative charge and migrate from the point of application toward the anode. When hemoglobin electrophoresis is performed on cellulose acetate at pH 8.6, hemoglobin A migrates the fastest toward the anode, followed respectively by hemoglobins F and S. Hemoglobins A_2 and C have the same electrophoretic mobility and migrate slightly slower than hemoglobin S. Because hemoglobins A_2 and C exhibit nearly the same mobility, they cannot be differentiated on cellulose acetate.

67.

D. At pH 6.2 on agar gel, hemoglobins exhibit different electrophoretic mobilities in comparison with hemoglobins electrophoresed at pH 8.6 on cellulose acetate. The order of migration of hemoglobins on cellulose acetate, proceeding from the most anodal hemoglobin to the most cathodal hemoglobin, is respectively A_1 and F, followed by G, D, and S, which migrate with the same mobility, followed by the group A_2, C, O, and E, which migrate the most slowly with the same mobility. This migration pattern is in contrast to agar gel electrophoresis at pH 6.2 in which the order of migration, from the most anodal hemoglobin to the most cathodal hemoglobin, is, respectively, C and S, followed by hemoglobins A_1, A_2, D, E, and G, which migrate as a group with the same mobility, followed by F. The different migration patterns seen with these two media systems are useful in differentiating hemoglobins that migrate with the same electrophoretic mobility. In the case of hemoglobins A_2 and C, which migrate with the same mobility on cellulose acetate, it is not possible to discern which hemoglobin is present in a particular blood specimen. By electrophoresing this specimen on agar gel at pH 6.2, hemoglobin A_2 may be differentiated from hemoglobin C because hemoglobin A_2 exhibits mobility similar to that of hemoglobin A_1, whereas hemoglobin C migrates alone closest to the anode.

68.

D. Although hemoglobin electrophoresis is the recommended method for hemoglobin identification, solubility testing may be warranted for large-scale screening for hemoglobin S. Solubility testing is possible because the solubility properties of most hemoglobins differ enough from those of hemoglobin S. In this method, sodium hydrosulfite acts as a reducing agent to deoxygenate hemoglobin. In the presence of hemoglobin S, the concentrated phosphate buffer test solution will become turbid because deoxygenated hemoglobin S is insoluble in the buffer solution. Hemoglobins A_1, C, D, and F, when present, will remain soluble in the phosphate buffer solution and show no visible signs of turbidity. Therefore, the detection of turbidity is associated with the presence of hemoglobin S.

69.

C. Since the conception of radioimmunoassay (RIA), in the early 1960s, a variety of immunoassay techniques have been developed and applied to measuring a wide variety of substances that are present in the blood in very small concentrations. Categories of ligands for which immunoassay methods have been developed include drugs, hormones, vitamins, tumor markers, and enzymes. Electrolytes are commonly quantified using ion-selective electrodes. Some drugs that are assayed by immunoassay include digoxin, gentamicin, phenobarbital, phenytoin, and theophylline. Immunoassay methods are available for the vitamins B_{12} and folic acid. Creatine kinase-MB isoenzyme mass analysis uses an immunoassay technique. The list of hormones that are assayed by immunoassay is extensive. Some of these hormones are thyroxine, triiodothyronine, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, estradiol, estriol, beta-chorionic gonadotropin, cortisol, prolactin, aldosterone, insulin, gastrin, testosterone, and prostaglandins. The immunoassay methods are generally automated, and enzyme labels and fluorogenic labels are commonly used.

70.

D. Enzyme-multiplied immunoassay technique (EMIT) is an example of a homogeneous immunoassay technique. A homogeneous assay is one in which separation of the bound and free fraction is unnecessary. The antigen is labeled with an enzyme and competes with the unknown antigen for binding sites on the antibody. The enzyme-labeled antigen that remains in the free fraction is enzymatically active. Therefore, the free labeled antigen can be determined by its action on a substrate in the presence of bound-labeled fraction. This type of assay is used commonly on automated instruments. The other techniques mentioned in the question, RIA, ELISA, and IRMA, are termed heterogeneous immunoassays because they require the physical separation of the bound from the free fraction before actual measurement.

71.

D. A number of immunoassay methods have been developed for the quantification of hormones, drugs, tumor markers, and other analytes that are present in small concentrations in the blood. The overall principle involved is the same. That is, the substance to be measured reacts with a specific macromolecule of limited binding capacity; frequently, this binder is an antibody. All these assays are similarly dependent on the closeness with which the unknown species and the standard react with the binder. These assays differ only in the specific reagents used. The ELISA system depends on enzyme-labeled antigen. Competitive protein binding (CPB) is a general term for any system that uses serum protein or tissue receptors for binding agents. Other methods based on antigen-antibody reactions, include such assays as fluorescent polarization immunoassay (FPI), enzyme-multiplied immunoassay technique, and chemiluminescence assays. Although hormones may be quantified using high-performance liquid chromatography, its principle is based on differential partitioning of compounds and not on antigen-antibody reactions as for the immunoassays.

72.

D. EMIT employs a homogeneous enzyme immunoassay method. This means that physical separation of the free labeled antigen from the antibody-bound-labeled antigen is not necessary for measurement. This is possible because only the free labeled antigen remains active. In the EMIT system the antigen is labeled with an enzyme (e.g., glucose-6-phosphate dehydrogenase). Determination of the drug concentration in the serum sample is made when the free enzyme-labeled drug reacts with substrate and coenzyme, resulting in an absorbance change that is measured spectrophotometrically. The drug in the serum sample is the unlabeled antigen in the assay, and it competes with the labeled drug for the binding sites on the antibody.

73.

B. The components needed in EMIT include the free unlabeled drug (unlabeled antigen) in the serum specimen, antibody specific to the drug being quantified, enzyme-labeled drug (labeled antigen), and substrate and coenzyme specific for the enzyme. In this method, the enzyme is coupled to the drug, producing an enzyme-labeled drug also referred to as an enzyme-labeled antigen. This enzyme-labeled complex competes with free unlabeled drug in the serum sample for the binding sites on the antibody. EMIT therapeutic drug monitoring assays are available for a variety of drugs that are included in the categories of antimicrobial, antiepileptic, antiasthmatic, cardioactive, and antineoplastic drugs. The EMIT system is not limited only to drug assays but is also available for hormone testing.

74.

C. In the EMIT assay, antibody specific to the drug being quantified is added to the serum sample that contains the drug. Substrate and coenzyme specific for the enzyme label being used are added. Finally, the enzyme-labeled drug (free labeled antigen) is added to the mixture. The drug in the serum sample and the enzyme-labeled drug compete for the binding sites on the antibody. The binding of the enzyme-labeled drug to the antibody causes a steric alteration that results in decreased enzyme activity. This steric change prevents the substrate from reacting at the active site of the enzyme, leaving only the free enzyme-labeled drug able to react with the substrate and coenzyme. The resulting enzyme activity, measured at 340 nm, is directly proportional to the concentration of the drug in the serum sample. The greater the amount of enzyme activity measured, the greater is the concentration of free enzyme-labeled drug and, therefore, the greater is the concentration of drug in the serum sample.

75.

D. Luminescent oxygen channeling immunoassay (LOCI™) is a homogeneous technique that is an adaptation of the chemiluminescent immunoassay. Singlet oxygen reacts with the precursor chemiluminescent compound to form a chemiluminescent product that decays and emits light. This light energy is accepted by a fluorophore, which results in light emission of a longer wavelength. In this assay, the chemiluminescent signal is enhanced by the resulting fluorescent signal which is proportional to the concentration of analyte in the serum sample.

76.

A. In a luminescent oxygen channeling immunoassay the antigen links to two antibody-coated particles. The first is an antibody-coated sensitizer particle containing a photosensitive dye (singlet oxygen source), and the second is an antibody-coated particle (singlet oxygen receptor) containing a precursor chemiluminescent compound and a fluorophore. Radiant energy is used to irradiate the immunocomplex, which stimulates the production of singlet oxygen at the surface of the sensitizer particle. The singlet oxygen diffuses to the second particle being held in close proximity.

Proteins and Tumor Markers

77.

D. The three major biochemical compounds that exert primary roles in human intermediary metabolism are proteins, carbohydrates, and lipids. The presence of nitrogen in all protein compounds distinguishes proteins from carbohydrates and lipids. Protein compounds contain approximately 16% nitrogen. Although there are only 20 common α-amino acids that are found in all proteins and a total of 40 known amino acids, a protein compound may contain from fifty to thousands of amino acids. The uniqueness of any protein is dictated by the number, type, and sequencing of the α-amino acids that compose it. The α-amino acids are linked to each other through peptide bonds. A peptide bond is formed through the linkage of the amino group of one amino acid to the carboxyl group of another amino acid.

78.

C. A variety of external factors, such as mechanical agitation, application of heat, and extreme chemical treatment with acids or salts, may cause the denaturation of proteins. When proteins are denatured, they undergo a change in their tertiary structure. *Tertiary structure* describes the appearance of the protein in its folded, globular form. When the covalent, hydrogen, or disulfide bonds are broken, the protein loses its shape as its polypeptide chain unfolds. With the loss of this tertiary structure, there is also a loss in some of the characteristic properties of the protein. In general, proteins will become less soluble, and enzymes will lose catalytic activity. Denaturation by use of chemicals has been a useful laboratory tool. The mixing of serum proteins with sulfosalicylic acid or trichloroacetic acid causes the precipitation of both the albumin and globulin fractions. When albumin is placed in water, dilute salt solutions, or moderately concentrated salt solutions, it remains soluble. However, the globulins are insoluble in water but soluble in weak salt solutions. Both the albumins and globulins are insoluble in concentrated salt solutions. *Primary structure* refers to the joining of the amino acids through peptide bonds to form polypeptide chains. *Secondary structure* refers to the twisting of more than one polypeptide chain into coils or helices.

79.

D. Although the Kjeldahl technique for the determination of protein nitrogen is too cumbersome for use in routine testing, it is considered to be the reference method of choice to validate materials used with the biuret method. The Kjeldahl technique is based on the quantification of the nitrogen content of protein. It is estimated that the average nitrogen content of protein is 16% of the total weight. In the Kjeldahl technique, protein undergoes a digestion process with sulfuric acid through which the nitrogen content of the protein is converted to ammonium ion. The ammonium ion in turn may be reacted with Nessler's reagent, forming a colored product that is read spectrophotometrically, or the ammonium ion may undergo distillation, liberating ammonia that is titrated.

80.

C. A commonly used method to quantify serum total proteins is the biuret procedure. The biuret reaction is based on the complexing of cupric ions in an alkaline solution with the peptide linkages of protein molecules. Because the amino acids of all proteins are joined together by peptide bonds, this method provides an accurate quantification of the total protein content of serum. The greater the amount of protein in a specimen, the greater will be the number of available peptide bonds for reaction and the more intense the colored reaction will be. In the biuret reaction, the intensity of the reddish violet color produced is proportional to the number of peptide bonds present. Generally, one cupric ion complexes with four to six peptide linkages. However, a colored product may be formed when the cupric ion links through coordinate bonds with at least two peptide linkages, with the smallest compound able to react being the tripeptide. Therefore, not only will proteins contribute to the formation of the colored product, but so, too, will any tripeptides and polypeptides present in a serum sample.

81.

B. The concentration of total protein in cerebrospinal fluid (CSF) is 15–45 mg/dL. Such a low level of protein requires a method with sufficient sensitivity such as Coomassie brilliant blue. Turbidimetric methods can also be used to quantify protein in CSF. Neither biuret nor Ponceau S has the sensitivity needed, and bromcresol green measures only albumin and does not react with the globulins.

82.

D. CSF, an ultrafiltrate of blood plasma, is made in the choroid plexus of the ventricles of the brain. Protein quantification is among the tests generally ordered on CSF; other tests include glucose, culture and sensitivity, and differential cell count. The reference range for CSF protein is 15–45 mg/dL. CSF protein may be quantified using turbidimetric (e.g., sulfosalicylic acid and benzethonium chloride) or dye-binding methods (e.g., Coomassie brilliant blue). Elevated levels of CSF protein are found in such disorders as bacterial, viral, and fungal meningitis; multiple sclerosis; neoplasm; disk herniation; and cerebral infarction. Low levels of CSF protein are found in hyperthyroidism and in CSF leakage from the central nervous system.

83.

D. Bisalbuminemia is a congenital disorder that does not exhibit any clinical manifestations. The only sign of this disorder is the splitting of albumin into two distinct bands when serum is subjected to electrophoresis. The extra albumin band may occur either anodically or cathodically to the normal albumin band depending on its speed of migration. The intensity of the two bands when quantified by densitometry may show that the two forms are of equal concentration. In a less common variation the abnormal albumin band may represent only 10–15% of the total albumin concentration.

84.

C. There are no physiological diseases that cause increased production of albumin by the liver. Elevated serum albumin is only associated with dehydration. It is a relative increase that will return to normal when fluids are administered to alleviate the dehydration. Disorders such as malnutrition, acute inflammation, and renal disease are characterized by decreased serum albumin levels.

85.

A. In renal disease, glomerular or tubular malfunction results in proteinuria. In early stages of glomerular dysfunction, small quantities of albumin will appear in the urine. Because the concentration is so low, urine dipstick assays are unable to detect the presence of such a small quantity of albumin; hence the term "microalbuminuria." Annual testing of diabetic individuals for microalbuminuria is recommended, because identification of these low levels of albumin that precede nephropathy would allow for clinical intervention to control blood glucose levels and blood pressure. The reference interval for urinary albumin is less than 30 mg/day. Microalbuminuria may be quantified using immunonephelometry and enzyme immunoassay.

86.

B. β_2-Microglobulin is a single polypeptide chain that is the light chain component of human leukocyte antigens (HLAs). It is found on the surface of nucleated cells and is notably present on lymphocytes. Increased plasma levels of β_2-microglobulin are associated with renal failure, lymphocytosis, rheumatoid arthritis, and systemic lupus erythematosus.

87.

A. Haptoglobin is a glycoprotein produced mainly by the liver that migrates electrophoretically as an alpha$_2$-globulin. Increased serum concentrations of haptoglobin are seen in inflammatory conditions and tissue necrosis, whereas decreased levels are seen in hemolytic situations in which there is extensive red blood cell destruction. In the latter situation, haptoglobin binds with free hemoglobin to form a stable complex that may then be removed by the reticuloendothelial system. Because of the size of the haptoglobin-hemoglobin complex, urinary excretion of hemoglobin by the kidney is avoided, thereby preventing the loss of iron by the kidney.

88.

D. The serum proteins are divided into five principal fractions based on their electrophoretic mobilities. The five fractions are albumin, alpha$_1$-globulin, alpha$_2$-globulin, beta-globulin, and gamma-globulin. Albumin constitutes the largest individual fraction of the serum proteins. The reference concentration of albumin in serum ranges between 3.5 and 5.0 g/dL, and the total globulin concentration is between 2.3 and 3.5 g/dL.

89.

C. Bromcresol green (BCG) and bromcresol purple (BCP) are anionic dyes that bind selectively with albumin without preliminary extraction of the globulins. The nature of the dyes is such that the color of the free dye is different from the color of the albumin-dye complex so that the color change is directly proportional to the concentration of albumin in the specimen. Although amido black, Ponceau S, and Coomassie brilliant blue are able to bind albumin, they also react with the globulins, thus prohibiting their use in a direct procedure for quantification of serum albumin.

90.

B. Biuret reagent is a combination of copper sulfate, potassium iodide in sodium hydroxide, and potassium sodium tartrate. The copper sulfate is the key to the reaction because it is the cupric ion that complexes with the peptide bonds of protein. To keep the copper in solution until its use, potassium sodium tartrate is employed as a complexing agent, whereas the autoreduction of copper is prevented by potassium iodide.

91.

D. The majority of the plasma proteins are manufactured by the liver. Albumin, fibrinogen, and most of the alpha- and beta-globulins are produced by the liver. The immunoglobulins, including IgG, IgA, IgM, IgD, and IgE, are produced by the lymphoid cells.

92.

D. The immunoglobulins, IgG, IgA, IgM, IgD, and IgE, migrate electrophoretically with the gamma-globulin fraction. The normal serum levels of the IgD and IgE classes are so low that these two immunoglobulins do not normally contribute to the intensity of the stained gamma-globulin electrophoretic fraction. The primary component of the gamma fraction consists of IgG, with IgA and IgM contributing to the intensity of the stained fraction to a lesser degree. In disease states the concentration relationship between the immunoglobulins may be significantly altered from the normal.

93.

D. All the immunoglobulins consist of heavy- and light-chain polypeptides. The heavy chains are designated as gamma γ, alpha α, mu μ, delta Δ, and epsilon ε and are specific for the immunoglobulins IgG, IgA, IgM, IgD, and IgE, respectively. The light chains are designated as kappa κ and lambda λ, with both types being found in each of the immunoglobulin classes, although the two light chains attached to a particular set of heavy chains must be of the same type. Therefore, IgG consists of two heavy chains of the gamma type and two light chains of either the kappa or lambda type. The immunoglobulins IgA, IgD, and IgE have a structure similar to that of IgG in that they consist of two light chains and two heavy chains of the respective type. IgM is a macromolecule with a pentamer type of structure. IgM consists of five sets of two heavy-chain and two light-chain units, with the basic units being linked to each other by peptide fragments.

94.

A. The immunoglobulin class IgA is found in both plasma and body secretions, with the two types being differentiated by their sedimentation coefficients. Plasma IgA has an average sedimentation coefficient of 7S, and secretory IgA has a sedimentation coefficient of 11S. Secretory IgA is present in saliva, tears, and secretions of nasal, gastrointestinal, and tracheolbronchial origin. Secretory IgA is dimeric in structure and possesses a glycoprotein secretory component attached to its heavy chains and a J polypeptide. The principal immunoglobulin found in secretions is IgA, with only trace amounts of IgG being present. The presence of IgM, IgD, or IgE in secretions has not been detected.

95.

D. The only immunoglobulin class that is able to cross the placenta from the mother's circulation to the fetus is IgG. Therefore, at birth, there is very little immunoglobulin present in the infant except for the maternal IgG. After birth, as the infant comes in contact with antigens, the levels of IgG, IgA, and IgM slowly increase.

96.

D. α_1-Antitrypsin is an acute-phase reactant protein whose concentration increases in response to inflammation. α_1-Antitrypsin inhibits the self-destruction of one's own tissue by forming inactive complexes with proteolytic enzymes. In this way the enzymes are inhibited, and tissue destruction through self-digestion is avoided. α_1-Antitrypsin has been found to have the highest concentration in serum of any of the plasma proteolytic inhibitors. It is an effective inhibitor of the enzymes chymotrypsin, plasmin, thrombin, collagenase, and elastase. The primary effect of α_1-antitrypsin may be seen in the respiratory tract and the closed spaces of the body where physiological pH values are maintained. α_1-Antitrypsin is least effective in the stomach and intestines.

97.

A. Ceruloplasmin, a metalloprotein, is the principal transport protein of copper in the plasma. In the plasma, copper is primarily bound to ceruloplasmin, with only very small amounts of copper bound to albumin or in a dialyzable free state. When subjected to an electric field, ceruloplasmin migrates as an alpha$_2$-globulin.

98.

C. The liver of a fetus and the yolk sac produce a protein known as α_1-fetoprotein (AFP). The concentration of AFP in the blood of a fetus reaches a maximum concentration at approximately 16 to 18 weeks gestation. Blood levels decline from this point and finally disappear approximately 5 weeks after birth. In cases of open spina bifida or anencephaly, the fetus leaks large amounts of AFP into the amniotic fluid. By means of an amniocentesis, the amount of AFP present in the amniotic fluid may be quantified by enzyme-labeled immunoassay and other immunoassay techniques.

99.

C. Fibronectin is an adhesive glycoprotein that functions with collagen to support cell adhesion. It is a normal constituent in the placenta and amniotic fluid. As labor begins, a change occurs in cell adhesion that affects the placenta and uterine wall. The level of fetal fibronectin increases in the secretions of the cervix and vagina. When this occurs prematurely, the increase in fetal fibronectin is used to predict risk of premature birth. Inhibin A, α_1-fetoprotein, human chorionic gonadotropin, and unconjugated estriol are used together in the quadruple test to assess risk for such disorders as Down syndrome.

100.

D. The immunoglobulins are composed of both heavy and light chains. In Bence Jones proteinuria, there is an overproduction of one type of light chain by a single clone of plasma cells. Therefore, the plasma cells produce either an excessive amount of kappa light chains or an excessive amount of lambda light chains. The light-chain type produced is in such abundance that the renal threshold is exceeded, resulting in the excretion of free light chains of the kappa or lambda type in the urine. The type of light chain excreted in the urine may be identified by performing immunoelectrophoresis on a concentrated urine specimen. In addition, immunoturbidimetric and immunonephelometric methods may also be used.

101.

C. In multiple myeloma there is an abnormal proliferation of plasma cells. These plasma cells produce a homogeneous immunoglobulin protein that stains as a well-defined peak in the gamma region. Because of the presence of this monoclonal protein, the serum total protein will be elevated. Bone destruction is commonly seen in this disorder, with the plasma cells forming densely packed groups in the lytic areas. Hypercalcemia is primarily the result of bone destruction.

102.

A. Immunonephelometric and immunoturbidimetric techniques are used to quantify specific immunoglobulin classes. Nephelometric techniques used to quantify the immunoglobulins are based on the measurement of light scatter by the antigen-antibody complexes formed. This method also calls for the comparison of unknowns with standards. Although radial immunodiffusion can be used to quantify the immunoglobulins, it is not a method of choice. Serum protein electrophoresis, immunoelectrophoresis, and isoelectric focusing cannot be used to quantify the immunoglobulins.

103.

C. Portal cirrhosis is a chronic disease of the liver in which fibrosis occurs as a result of tissue necrosis and diffuse small nodules form as liver cells regenerate, with a concomitant distortion of liver structure. The cause of this disorder may include alcoholism, malnutrition, or submassive hepatic necrosis. When a serum protein electrophoresis is performed, the characteristic pattern seen in portal cirrhosis is an elevation of both the gamma- and beta-globulin regions, with these two regions showing a bridging or fusing appearance. This beta-gamma bridging effect is due to an increased level of IgA, which migrates with beta mobility. It should also be noted that the albumin level is depressed.

104.

D. Although microbiological analysis and chemical analysis may be employed to detect and quantify a specific amino acid, chromatographic analysis is preferred as a screening technique for amino acid abnormalities or when differentiation among several amino acids is necessary. Thin-layer chromatography, either one- or two-dimensional, is being used in conjunction with a mixture of ninhydrin-collidine for color development. To quantify amino acids high-performance liquid chromatography, ion-exchange chromatography, and tandem mass spectrometry are used.

105.

C. Protein electrophoresis is performed on a serum specimen. If plasma is substituted for serum, the electrophoresis will show an extra fraction in the beta-gamma region, because fibrinogen is a $beta_2$-globulin. This extra fraction represents the protein fibrinogen that is present in a plasma specimen. Fibrinogen contributes approximately 0.2–0.4 g/dL to the total protein concentration.

106.

A. When serum proteins are exposed to a buffer solution of pH 8.6, the proteins take on a net negative charge. The negatively charged proteins will migrate toward the anode (+) when exposed to an electrical field. Albumin migrates the fastest toward the anode whereas the gamma-globulins remain close to the point of application and actually move slightly in a cathodic (–) direction because of the effects of endosmosis. The order of migration of the serum proteins, starting at the anode with the fastest-moving fraction, is albumin, alpha$_1$-globulin, alpha$_2$-globulin, beta-globulin, and gamma-globulin.

107.

D. α_1-Fetoprotein, synthesized by the fetus, peaks at 13 weeks and declines at 34 weeks of gestation. When concern exists for the well-being of the fetus, maternal serum AFP is measured between 15 and 20 weeks of gestation. An increased AFP level in maternal serum is associated with such disorders as neural tube defects, spina bifida, and fetal distress. A decreased AFP level in maternal serum is characteristic of Down syndrome.

108.

C. The normal range for total PSA is sometimes referenced as less than 4.0 ng/mL. Early detection guidelines endorse a lower cutoff for total PSA up to 2.5 ng/mL and recommend that values >2.5 ng/mL should be followed up by performing a biopsy. Men with prostate cancer tend to have lower % free PSA (free PSA/total PSA) than men with benign disease; thus lower % free PSA is associated with a higher risk of prostate cancer. In the case presented, the patient's total PSA was 3.1 ng/mL with a free PSA of 0.3 ng/mL, which is 10% free PSA. This low percentage is suggestive of a higher probability of cancer, whereas a percentage >25% is associated with lower risk of cancer.

109.

B. Carcinoembryonic antigen (CEA), a glycoprotein, is found in increased amounts in serum when malignant tumors of the colon, lung, pancreas, stomach, and breast are present. Care must be exercised in treating CEA as a diagnostic test, because elevated values are also seen in smokers, hepatitis patients, and patients with several other nonmalignant disorders. Clinically, CEA is more valuable in prognosis and treatment monitoring. Enzyme immunoassay and other types of immunoassays are available for the quantification of CEA.

110.

B. AFP is normally produced only by the fetus, with blood levels disappearing shortly after birth. However, in the adult, such conditions as hepatoma or teratoma stimulate the production of this primitive protein by the tumor cells. The quantification of AFP may be used both diagnostically and as a monitor of chemotherapy.

111.

D. PSA is a single-chain glycoprotein whose function aids in the liquefaction of seminal coagulum. PSA is found specifically in the prostate gland, and elevated levels are associated with prostate cancer and benign prostatic hyperplasia (BPH). Thus, combining the quantification of PSA with the performance of the digital rectal examination is more beneficial for prostate cancer detection. Immunoassays using enzyme, fluorescent, and chemiluminescent labels are available to quantify PSA.

112.

B. CA 125 is an oncofetal antigen, glycoprotein in nature, that is produced by ovarian epithelial cells. The majority of individuals with nonmucinous epithelial ovarian cancer exhibit elevated levels of CA 125. CA 125 is also increased in other malignancies, including endometrial, breast, colon, pancreas, and lung cancers. Several benign disorders also exhibit CA 125 elevated levels. It appears that the primary usefulness of CA 125 is in monitoring the success of therapy in treating ovarian carcinoma.

113.

A. CA 19-9 is an oncofetal protein that is a sialylated Lewis blood group antigen. It is found in increased levels in colorectal carcinoma as well as in gastric, hepatobiliary, and pancreatic cancers. CA 19-9 is also elevated in several benign disorders, including pancreatitis, extra-hepatic cholestasis, and cirrhosis. The combination use of CA 19-9 and CEA (carcinoembryonic antigen) is helpful in monitoring the recurrence of colorectal cancer.

114.

B. Elevations of serum levels of AFP are found in a number of malignant as well as benign disorders. Although AFP is considered the most specific laboratory test for hepatocellular carcinoma, increased levels are also found in benign liver disease, including viral hepatitis, chronic active hepatitis, and cirrhosis. Other malignant disorders associated with increased levels of AFP include testicular and ovarian germ cell tumors, pancreatic carcinoma, gastric carcinoma, and colonic carcinoma. Thus, AFP is not a tissue-specific tumor marker. AFP is not elevated in prostatic cancer, which is characterized by an elevation in PSA. The use of AFP in conjunction with human chorionic gonadotropin (hCG) is effective in monitoring treatment and identifying recurrence of testicular cancer.

115.

D. hCG is a dimer consisting of alpha and beta polypeptide chains, with the β subunit conferring immunogenic specificity. Although hCG is more commonly associated with testing to confirm pregnancy, it is also associated with certain forms of cancer. β-hCG is used as a tumor marker for hydatidiform mole, gestational choriocarcinoma, and placental-site trophoblastic tumor. hCG's utility also extends to monitoring the success of therapy in testicular and ovarian germ cell tumors. In addition, increased levels of hCG have been identified in hematopoietic malignancy, melanoma, gastrointestinal tract neoplasms, sarcoma, and lung, breast, and renal cancers.

116.

D. CA 15-3 and CA 549 are oncofetal antigens that are glycoprotein in nature. CA 15-3 is found on mammary epithelium. Increased serum levels of CA 15-3 are found in breast, pancreatic, lung, colorectal, and liver cancers. CA 549 is found in the cell membrane and luminal surface of breast tissue. Increased serum levels of CA 549 are found in breast, lung, prostate, and colon cancers. Although both CA 15-3 and CA 549 are elevated in more advanced stages of breast cancer, neither is helpful in detecting early stages of breast cancer.

Nonprotein Nitrogenous Compounds

117.

D. Constituents in the plasma that contain the element nitrogen are categorized as being protein- or nonprotein-nitrogen compounds. The principal substances included among the nonprotein-nitrogen compounds are urea, amino acids, uric acid, creatinine, creatine, and ammonia. Of these compounds, urea is present in the plasma in the greatest concentration, comprising approximately 45% of the nonprotein-nitrogen fraction.

118.

D. Because the substances classified as nonprotein-nitrogen (NPN) compounds were quantified by assaying for their nitrogen content, it became customary to express urea as urea nitrogen. When urea was expressed as urea nitrogen, a comparison could be made between the concentration of urea and the concentration of other NPN compounds. When it is necessary to convert urea nitrogen values to urea, the concentration may be calculated easily by multiplying the urea nitrogen value by 2.14. This factor is derived from the molecular mass of urea (60 daltons) and the molecular weight of its two nitrogen atoms (28):

$$\frac{60}{28} = 2.14$$

119.

B. In addition to the fact that sodium fluoride is a weak anticoagulant, it also functions as an antiglycolytic agent and is used as a preservative for glucose in blood specimens. With the urease reagent systems for the quantification of urea, the use of sodium fluoride must be avoided because of its inhibitory effect on this system. Additionally, contamination from the use of ammonium oxalate and ammonium heparin must be avoided, because urease catalyzes the production of ammonium carbonate from urea. In several methods, the ammonium ion formed reacts proportionally to the amount of urea originally present in the sample. Anticoagulants containing ammonium would contribute falsely to the urea result.

120.

B. In the diacetyl method, acidic diacetyl reacts directly with urea to form a yellow-diazine derivative. Thiosemicarbazide and ferric ions are reagents used to intensify the color of the reaction. Because urea is quantified directly, the method does not suffer from interferences from ammonia contamination, as do some of the urea methods.

121.

A. Adequate specificity is generally obtained when using the urease/glutamate dehydrogenase method. Because urease hydrolyzes urea to ammonia and water, a positive interference from endogenous ammonia will occur with elevated blood levels of ammonia. Such interference may occur from use of aged blood specimens and in certain metabolic diseases.

122.

B. An enzymatic method for quantifying urea employs urease and glutamate dehydrogenase (GLDH) in a coupled enzymatic reaction. Urease catalyzes the production of ammonium carbonate from urea. The ammonium ion produced reacts with 2-oxoglutarate and NADH in the presence of GLDH with the formation of NAD^+ and glutamate. The decrease in absorbance, as NADH is oxidized to NAD^+, is followed kinetically at 340 nm using a spectrophotometer. In the conductimetric method, the formation of ammonium ions and carbonate ions, from the ammonium carbonate, causes a change in conductivity that is related to the amount of urea present in the sample.

123.

C. The Berthelot reaction is based on the production of a blue-indophenol compound when ammonia reacts in an alkaline medium with phenol and sodium hypochlorite. This basic colorimetric reaction can be used to quantify both urea and blood ammonia levels. Therefore, any ammonia contamination (i.e., in the distilled water used to make reagents for the urea procedure and on glassware) must be avoided so that falsely elevated urea values will not be obtained.

124.

A. The catabolism of some amino acids involves a transamination reaction in which the α-amino group of the amino acid is enzymatically removed. After its removal, the α-amino group is transferred to an α-keto acid (α-ketoglutarate) with the formation of L-glutamate. Glutamate, which is the common product formed by most transaminase reactions, then may undergo oxidative deamination in the liver mitochondria with the formation of ammonia. The ammonia thus formed leaves the mitochondria as the amino group of citrulline. Citrulline, in turn, condenses with aspartate, which contains the second amino group needed for urea synthesis, forming argininosuccinate, which ultimately leads to the formation of urea. Therefore, the formation of urea and its excretion in the urine provide the principal means by which the body is able to free itself of excess ammonia.

125.

D. It is necessary that certain precautions in specimen handling be exercised because the enzymatic process of deamination of amides continues at room temperature after a blood sample is drawn. When blood is drawn for ammonia analysis, it is critical that any *in vitro* ammonia formation be prevented. It is recommended that the tube containing the blood specimen be placed in an ice bath immediately after the blood is drawn, because the cold environment will help retard metabolic processes. It is also important that the chemical analysis of the specimen be started within 20 minutes of drawing the specimen.

126.

C. Plasma is the specimen of choice for ammonia analysis. Ethylenediaminetetra-acetic acid (EDTA) and heparin (not the ammonium salt) are acceptable anticoagulants. Because exposure of blood to air is contraindicated, the evacuated blood collection tube should be filled completely. The blood specimen should be placed on ice immediately and centrifuged as soon as possible to inhibit deamination of amino acids. Because the concentration of ammonia in red blood cells is approximately three times greater than in plasma, the analysis should be performed on a nonhemolyzed specimen. Because of the false increase in ammonia levels caused by smoking, patients should be instructed to refrain from smoking for 8 hours before blood collection.

127.

D. Ion-exchange, ion-selective electrode, and enzymatic methods have been employed for the analysis of ammonia in plasma specimens. Because the enzymatic method is a direct assay, prior separation of ammonium ions is not required. The enzymatic reaction catalyzed by glutamate dehydrogenase follows:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADPH} \rightleftarrows \text{Glutamate} + NADP^+ + H_2O$$

The rate of oxidation of NADPH to $NADP^+$ is followed as a decreasing change in absorbance at 340 nm.

128.

D. The gastrointestinal tract is the primary source of blood ammonia. With normal liver function, ammonia is metabolized to urea for urinary excretion. When blood ammonia levels become elevated, toxicity of the central nervous system occurs. Diseases associated with elevated blood ammonia levels include Reye syndrome, renal failure, chronic liver failure, cirrhosis, and hepatic encephalopathy.

129.

A. Creatinine is a waste product of muscle metabolism and as such its production is rather constant on a daily basis. Creatinine is freely filtered by the glomerulus, with only a very small amount secreted by the proximal tubule. Thus, measurement of creatinine is a reflection of glomerular filtration. An increase in the serum creatinine level would be indicative of decreased glomerular filtration. Although uric acid, urea, and ammonia levels may be increased with decreased glomerular filtration, increased levels of these analytes are associated with a number of specific metabolic diseases and, therefore, they are not used as indicators of the glomerular filtration rate.

130.

D. Serum urea nitrogen and creatinine levels are frequently requested together so that their ratio can be evaluated. The normal ratio of serum urea nitrogen to creatinine ranges between 10:1 and 20:1. Abnormal values obtained when kidney function tests are performed may be the result of a prerenal, renal, or postrenal malfunction. The ratio of urea nitrogen to creatinine is sometimes used as an index in the assessment of kidney function and as a means of differentiating the source of the malfunction.

131.

C. Creatine is synthesized from the amino acids arginine, glycine, and methionine. In tissues that include the kidneys, small intestinal mucosa, pancreas, and liver, arginine and glycine form guanidoacetate through a transaminidase reaction. The guanidoacetate is transported in the blood to the liver, where it reacts with *S*-adenosylmethionine through a transmethylase reaction to form creatine. Creatine is transported in the blood to muscle tissue. Creatine in the form of phosphocreatine is a high-energy storage compound that provides the phosphate needed to produce adenosine triphosphate (ATP) for muscle metabolism. When ATP is formed from phosphocreatine, free creatine is also released. Creatine, through a spontaneous and irreversible reaction, forms creatinine. Creatinine serves no functional metabolic role. It is excreted in the urine as a waste product of creatine.

132.

D. The Jaffe reaction, which was described in 1886, is still used for creatinine analysis. The Jaffe reaction employs the use of an alkaline picrate solution that reacts with creatinine to form a bright orange-red complex. A drawback to this procedure is its lack of specificity for creatinine, because noncreatinine chromogens, glucose, and proteins are also able to react with alkaline picrate.

133.

A. Because protein will interfere with the Jaffe reaction, serum for a manual creatinine analysis is treated with sodium tungstate and sulfuric acid to precipitate the proteins. The use of tungstic acid to make a protein-free filtrate is known as the Folin-Wu method. The protein-free filtrate, which still contains creatinine and other reducing substances, is then mixed with alkaline picrate reagent to yield the characteristic Jaffe reaction. Automated methods have replaced manual methods. These kinetic methods using the alkaline picrate reagent system have been adapted to use small volumes of serum and have readings taken within a short interval of 25–60 sec following initiation of the reaction. Because of the speed at which the analysis is performed and the small serum sample requirement, serum may be used directly, alleviating the need for a protein-free filtrate.

134.

D. The creatinine clearance test is used to assess the glomerular filtration rate. An accurately timed 24-hour urine specimen and a blood sample, drawn in the middle of the 24-hour urine collection, are required. The creatinine concentrations of the urine specimen and the plasma are determined, and these values, along with the urine volume, are used to determine the creatinine clearance. The body surface area will not be used in the calculation because the clearance is being done on an average-size adult. The following general mathematical formula is used to calculate creatinine clearance:

$$\frac{U}{P} \times V = \text{Creatinine clearance (mL/min)}$$

where U = urine creatinine concentration in milligrams per deciliter, P = plasma creatinine concentration in milligrams per deciliter, and V = volume of urine per minute, with volume expressed in milliliters and 24 hours expressed as 1440 minutes. Applying this formula to the problem presented in the question:

$$\frac{120 \text{ mg/dL}}{1.2 \text{ mg/dL}} \times \frac{1520 \text{ mL/24 hr}}{1440 \text{ min/24 hr}} = 106 \text{ mL/min}$$

It should be noted that both the size of the kidney and the body surface area of an individual influence the creatinine clearance rate. Because normal values for creatinine clearance are based on the average adult body surface area, it is necessary that the clearance rate be adjusted when the body surface area of the individual being tested differs significantly from the average adult area. This type of adjustment is especially critical if the individual is an infant, a young child, or an adolescent. The body surface area may be calculated from an individual's height and weight, or it may be determined from a nomogram. The average body surface area is accepted as being 1.73 m^2. The mathematical formula used to calculate a creatinine clearance when the body surface area of the individual is required follows:

$$\frac{U}{P} \times V \times \frac{1.73}{A} = \text{Creatinine clearance (mL/min/standard surface area)}$$

where 1.73 = standard adult surface area in square meters and A = body surface area of the individual in square meters.

135.

C. Creatinine assays are preferably performed on fresh urine specimens. If an acid urine specimen is kept for a time, any creatine in the urine will be converted to creatinine. In alkaline urine, an equilibrium situation will occur between the creatine and creatinine present in the specimen. To avoid either of these situations, it is recommended that the urine be adjusted to pH 7.0 and that the specimen be frozen. It is thought that at a neutral pH, the integrity of the urine specimen will be maintained because it will require days or even weeks for equilibrium to occur between the two compounds.

136.

D. Creatine is predominantly found in muscle cells, where the quantity of creatine is proportional to muscle mass. As muscle metabolism proceeds, creatine is freed from its high-energy phosphate form, and the creatine, thus liberated, forms the anhydride creatinine. The quantity of creatinine formed daily is a relatively constant amount because it is related to muscle mass. Therefore, it has been customary to quantify the creatinine present in a 24-hour urine specimen as an index of the completeness of the collection.

137.

A. In addition to the endpoint and kinetic methods, which use the Jaffe reaction (picric acid), several methods have been developed that use coupled enzymatic reactions for the quantification of creatinine. In one such method, creatinine amidohydrolase (creatininase) catalyzes the conversion of creatinine to creatine and subsequently to sarcosine and urea. Sarcosine oxidase catalyzes the oxidation of sarcosine to glycine, formaldehyde, and hydrogen peroxide. The hydrogen peroxide reacts with the reduced form of a chromogenic dye in the presence of peroxidase to form an oxidized colored dye product that is read spectrophotometrically.

138.

C. Creatinine is an endogenous substance that is filtered by the glomeruli and normally is neither reabsorbed nor secreted by the tubules. When plasma levels of creatinine rise, some secretion of creatinine by the tubules will occur. The filtration properties of creatinine and the fact that it is a substance normally present in blood make the creatinine clearance test the method of choice for assessing the glomerular filtration rate.

139.

B. Through a sequence of enzymatic reactions, the purine nucleosides, adenosine and guanosine, are catabolized to the waste product uric acid. The catabolism of purines occurs primarily in the liver, with the majority of uric acid being excreted as a urinary waste product. The remaining amount of uric acid is excreted in the biliary, pancreatic, and gastrointestinal secretions through the gastrointestinal tract. In the large intestine, uric acid is further degraded by bacteria and excreted in the stool.

140.

D. Uric acid may be quantified by reacting it with phosphotungstic acid reagent in alkaline solution. In this reaction, uric acid is oxidized to allantoin and the phosphotungstic acid is reduced, forming a tungsten blue complex. The intensity of the tungsten blue complex is proportional to the concentration of uric acid in the specimen.

141.

B. Uric acid absorbs light in the ultraviolet region of 290–293 nm. When uricase is added to a uric acid mixture, uricase destroys uric acid by catalyzing its degradation to allantoin and carbon dioxide. On the basis of these two characteristics, differential spectrophotometry has been applied to the quantification of uric acid. This type of method is used on analyzers that are capable of monitoring the decrease in absorbance as uric acid is destroyed by uricase. The decrease in absorbance is proportional to the concentration of uric acid in the specimen.

142.

A. As renal function continues to be lost over time, chronic renal failure develops. Chronic renal failure is manifested by loss of excretory function, inability to regulate water and electrolyte balance, and increased production of parathyroid hormone, all of which contribute to the abnormal laboratory findings. The decreased production of erythropoietin causes anemia to develop.

143.

D. Gout is a pathological condition that may be caused by a malfunction of purine metabolism or a depression in the renal excretion of uric acid. Two of the major characteristics of gout are hyperuricemia and a deposition of uric acid as monosodium urate crystals in joints, periarticular cartilage, bone, bursae, and subcutaneous tissue. Such a deposition of urate crystals causes inflammation of the affected area and precipitates an arthritic attack.

144.

A. An increase in serum uric acid levels may be seen during chemotherapy for leukemia. The cause of this is the accelerated breakdown of cell nuclei in response to the chemotherapy. Other proliferative disorders that may respond similarly are lymphoma, multiple myeloma, and polycythemia. It is important that serum uric acid be monitored during chemotherapy to avoid nephrotoxicity.

Carbohydrates

145.

D. When two monosaccharides condense with loss of a molecule of water, a disaccharide is formed. Disaccharides, therefore, can be hydrolyzed into two monosaccharides. The most important disaccharides are maltose, lactose, and sucrose. On hydrolysis, sucrose will yield one molecule of glucose and one molecule of fructose. Maltose can be hydrolyzed into two molecules of glucose. Lactose can be hydrolyzed into glucose and galactose.

146.

A. Glycogen is a polysaccharide composed of many glucose molecules. In contrast to the amylopectin molecule, a glycogen molecule is more highly branched and more compact. Glycogen is found in a variety of animal tissues, particularly in the liver, and provides the storage form for carbohydrates in the body. When energy requirements warrant it, glycogen may be broken down to glucose by a series of phosphorylating and related enzymes.

147.

A. There are three major classifications of carbohydrates: monosaccharides, disaccharides, and polysaccharides. Starch is classified as a polysaccharide because its structure is composed of many molecules of glucose (a monosaccharide) condensed together. Monosaccharides (e.g., glucose) are carbohydrates with the general molecular formula $C_n(H_2O)_n$ that cannot be broken down to simpler substances by acid hydrolysis. Disaccharides (e.g., sucrose, lactose) are condensation products of two molecules of monosaccharides with loss of one molecule of water.

148.

A. The level of glucose in the blood is a result of a variety of metabolic processes. Processes that increase the blood glucose include ingestion of sugar, synthesis of glucose from noncarbohydrate sources, and breakdown of glycogen. Processes that decrease blood glucose include metabolizing glucose to produce energy and converting glucose to glycogen or fat. Glycogen is a polysaccharide, which is the storage form of carbohydrates in animals. *Glycogenesis* refers to the formation of glycogen in the liver from blood glucose. This occurs in response to increased blood glucose levels. In response to decreasing blood glucose levels, glycogen in the liver is broken down to glucose. This process is called *glycogenolysis*. When glucose is metabolized, for example, to produce energy, it is converted to lactate or pyruvate. This process is called *glycolysis*. When the body synthesizes glucose from noncarbohydrate sources—that is, amino acids, glycerol, or lactate—the process is called *gluconeogenesis*. When the body uses glucose to synthesize fat, this process is called *lipogenesis*.

149.

A. When highly specific analytical methods are used, the glucose concentration in fasting whole blood is approximately 12–15% lower than in plasma or serum. Although glucose diffuses freely between the water phase of plasma and red blood cells, there is a higher concentration of water in plasma (approximately 12%) than in whole blood, accounting for the increased glucose concentration in plasma. The water content of whole blood depends on the hematocrit.

150.

D. Renal threshold is defined as the plasma level that must be exceeded in order for the substance to appear in the urine. The renal threshold for glucose is 180 mg/dL. This means that the blood glucose level must exceed 180 mg/dL in order for glucose to be excreted in the urine.

151.

D. *Glycated hemoglobin* is a collective term encompassing the three glycated hemoglobin fractions—hemoglobin A_{1a}, hemoglobin A_{1b}, and hemoglobin A_{1c}. Hb A_{1c} is the fraction of Hb A_1 that is present in the greatest concentration. Some commercially available column chromatography methods measure the three fractions collectively. *Glycated hemoglobin* refers to the specific red cell hemoglobin A types to which a glucose molecule becomes irreversibly attached. The greater the glucose concentration in the plasma, the greater the number of hemoglobin molecules that will become glycated. Because red blood cells have an average life span of 120 days and the glycation is irreversible, measurement of glycated hemoglobin reflects the average plasma glucose level of an individual during the previous 2- to 3-month period. This test is used as a monitor of diabetic control.

152.

B. The patient presents as having diabetes mellitus. The American Diabetes Association (ADA) published updated standards in 2007 for the classification and diagnosis of diabetes mellitus. Three criteria have been defined, with only one needing to be present to establish the diagnosis of diabetes mellitus. The three criteria include classic diabetic symptoms and a casual plasma glucose of ≥200 mg/dL, a fasting plasma glucose of ≥126 mg/dL, and a 2-hour postload plasma glucose (part of OGTT) of ≥200 mg/dL. It is recommended that any positive test be repeated on a subsequent day, if possible, to confirm the diagnosis. It should be noted that the OGTT is not recommended for routine clinical use and would be used only in special circumstances.

153.

C. Increased insulin resistance is commonly seen in the late second and third trimesters of pregnancy. Most women are able to compensate by secreting additional insulin and, thus, are able to maintain normal blood glucose levels. In cases of gestational diabetes mellitus, women are unable to make sufficient insulin to meet their needs. In the screening test, serum glucose is assessed at 1 hour following the ingestion of a 50-gram glucose load (glucose challenge test). If the serum glucose is ≥140 mg/dL, the next step is to perform an oral glucose tolerance test.

154.

D. Sodium fluoride is a weak anticoagulant that acts as a preservative for glucose. It functions as a glucose preservative by inhibiting glycolysis. However, it is not suitable for use with many enzyme procedures. In the determination of BUN, where urease activity is utilized, the high concentration of fluoride in the plasma acts as an enzyme inhibitor, preventing the necessary chemical reaction.

155.

D. Based on the biochemistry of the disease, diabetes mellitus has been classified as type 1 and type 2. Type 1 occurs more commonly in individuals under 20 years of age. Studies suggest that type 1 is associated with autoimmune destruction of β-cells, and it is characterized by insulin deficiency and thus a dependency on injection of insulin. Unlike people afflicted with type 2, type 1 individuals are prone to ketoacidosis and to such complications as angiopathy, cataracts, nephropathy, and neuropathy.

156.

C. The protein hormone insulin is synthesized in the pancreas by the β-cells of the islets of Langerhans. Insulin, a two-chain polypeptide, consists of 51 amino acids. A single-chain preproinsulin is cleaved to proinsulin, which is the immediate precursor of insulin. Proinsulin is hydrolyzed to form insulin, a two-chain polypeptide, and inactive C-peptide. Insulin promotes the entry of glucose into tissue cells.

157.

D. Insulin may be described as an anabolic, polypeptide hormone. Insulin stimulates glucose uptake by muscle cells (which increases protein synthesis), by fat cells (which increases triglyceride synthesis), and by liver cells (which increases lipid synthesis and glycogenesis). If cellular uptake of glucose is stimulated, the glucose concentration in the circulation decreases.

158.

D. In uncontrolled diabetes mellitus, the blood glucose level exceeds the renal threshold of approximately 180 mg/dL for glucose, leading to glycosuria and polyuria. The excess secretion of glucagon stimulates lipolysis, with increased formation of acetoacetic acid. In the blood, the ketoacids dissociate, with the hydrogen ions being buffered by bicarbonate. This causes the bicarbonate to become depleted and leads to metabolic acidosis.

159.

D. Glucose determinations are generally performed on serum or plasma rather than whole blood. Serum or plasma is more convenient to use than whole blood in most automated systems because serum does not require mixing before sampling. Glucose stability is greater in separated plasma than in whole blood because glycolysis is minimized. Specificity for glucose is higher when plasma or serum is used because variations attributable to interfering substances in the red cells are avoided.

160.

D. Research has demonstrated that there is a correlation between blood glucose levels in diabetes mellitus and the development of long-term complications. These complications may include such disorders as retinopathy, neuropathy, atherosclerosis, and renal failure. Thus, quantifying such blood analytes as urea, creatinine, and lipids as well as urinary albumin can aid in monitoring diabetic individuals.

161.

D. There are greater than 100 causes of hypoglycemia. Among the causes is the ingestion of certain drugs. Use of ethanol, propranolol, and salicylate has been linked to the occurrence of hypoglycemia.

162.

B. The diagnostic test for hypoglycemia is the 72-hour fast, which requires the analysis of glucose, insulin, C-peptide, and proinsulin at 6-hour intervals. The test should be concluded when plasma glucose levels drop to ≤45 mg/dL, when hypoglycemic symptoms appear, or after 72 hours have elapsed. In general, hypoglycemic symptoms occur when the plasma glucose level falls below 55 mg/dL. Such symptoms may include headache, confusion, blurred vision, dizziness, and seizures. The term "neuroglycopenia" has been applied to these central nervous system disorders. Although decreased hepatic glucose production and increased glucose utilization may cause hypoglycemia, there are over 100 causes of this disorder.

163.

B. Glucose in the presence of oxygen is oxidized to gluconic acid and hydrogen peroxide. This reaction is catalyzed by glucose oxidase. By using a polarographic oxygen electrode, the rate of oxygen consumption is measured and related to the concentration of glucose in the sample.

164.

C. The hexokinase method for quantifying glucose uses two coupled enzymatic reactions. In the first reaction, which is catalyzed by hexokinase, glucose is phosphorylated by adenosine triphosphate, forming glucose-6-phosphate and adenosine diphosphate. In the second reaction, glucose-6-phosphate dehydrogenase (derived from yeast) catalyzes the oxidation of glucose-6-phosphate and the reduction of nicotinamide adenine dinucleotide phosphate. The amount of reduced NADPH formed is proportional to the glucose concentration in the sample. Thus, the greater the absorbance reading of NADPH at 340 nm, the greater is the glucose concentration. If bacterial G-6-PD is used, the cofactor is NAD^+ with the production of NADH.

165.

A. The glucose oxidase method for quantifying glucose employs two coupled enzymatic reactions. In the first reaction, which is catalyzed by glucose oxidase, glucose in the presence of oxygen is oxidized to gluconic acid and hydrogen peroxide. In the second reaction, peroxidase catalyzes a reaction between hydrogen peroxide and the reduced form of a chromogenic oxygen acceptor, such as *o*-dianisidine, forming an oxidized colored product that is read spectrophotometrically.

166.

C. The glucose dehydrogenase method uses only one enzymatic reaction for the measurement of glucose in a sample. Glucose dehydrogenase catalyzes the oxidation of glucose and the reduction of nicotinamide adenine dinucleotide. The amount of reduced NADH formed is proportional to the glucose concentration in the sample. When measuring blood glucose levels during the administration of an oral xylose tolerance test, the glucose dehydrogenase method should not be used, because the relative rate of reaction of D-xylose as compared to glucose is 15% with this method. In contrast, D-xylose will not react in the hexokinase and glucose oxidase methods, thus allowing glucose to be measured accurately.

The D-xylose absorption test is useful in distinguishing two types of malabsorption: intestinal malabsorption and malabsorption resulting from pancreatic insufficiency. When D-xylose is administered orally, it is absorbed by passive diffusion into the portal vein from the proximal portion of the small intestine. Because D-xylose is not metabolized by the liver, it is excreted unchanged by the kidneys. In intestinal malabsorption, the amount of D-xylose excreted, as measured in a 5-hour urine specimen, is less than normal because of decreased absorption of D-xylose. In malabsorption caused by pancreatic insufficiency, the absorption of D-xylose is normal.

167.

C. Although there are several reliable enzymatic glucose methods available, the hexokinase method is the reference method for quantifying glucose. The reference method requires that a protein-free filtrate be made using barium hydroxide and zinc sulfate. The clear supernatant is then used as the sample in the hexokinase/glucose-6-phosphate dehydrogenase coupled enzyme reactions. For routine clinical use, serum is used directly in the hexokinase method because deproteinization is too time-consuming.

168.

C. The reference interval for glucose in CSF is 60% of the normal plasma value. For a plasma glucose of 110 mg/dL, the expected CSF glucose level would be 66 mg/dL. The equilibration of CSF with plasma glucose takes several hours. The reference interval for the CSF glucose level is 40–70 mg/dL as compared with a normal fasting plasma glucose level. Low levels of CSF glucose are associated with a number of diseases including bacterial meningitis and tuberculous meningitis, whereas viral disease generally presents with a normal level of CSF glucose.

169.

C. The reference interval for fasting serum glucose in an adult expressed in conventional units is 74–99 mg/dL. To convert conventional units to SI units (Système International d'Unités), multiply the conventional units in mg/dL by the 0.0555 conversion factor to obtain SI units in mmol/L. Thus, 74 mg/dL × 0.0555 = 4.1 mmol/L and 99 mg/dL × 0.0555 = 5.5 mmol/L. Although conventional units are used commonly in the United States, many scientific journals require the use of SI units in their publications and many foreign countries use SI units routinely in clinical practice. To identify additional conversion factors for other analytes, consult the appendix of a clinical chemistry textbook.

170.

B. It is currently recommended by the ADA that hemoglobin A_{1c} should be lowered to an average of approximately 7% in individuals with diabetes mellitus. When hemoglobin A_{1c} is reduced to this level or less, there is a reduction in microvascular and neuropathic complications of diabetes and to some degree macrovascular disease. Therefore, the ADA recommends that nonpregnant adults be maintained at a hemoglobin A_{1c} level of $<7\%$. There is some discussion that 6% would be better. Hemoglobin A_{1c} is the major component of the glycated hemoglobins. Quantification of hemoglobin A_{1c} may be performed using high-performance liquid chromatography, ion-exchange chromatography (manual), isoelectric focusing, and immunoassay techniques.

171.

D. Regulation of the blood glucose concentration depends on a number of hormones. These include insulin, glucagon, cortisol, epinephrine, growth hormone, adrenocorticotropic hormone, and thyroxine. Of these hormones, *insulin* is the only one that decreases the blood glucose level. *Glucagon* is produced in the pancreas by the alpha cells. Glucagon promotes an increase in the blood glucose concentration by its stimulatory effect on glycogenolysis in the liver. *Cortisol* is produced by the adrenal cortex. It stimulates gluconeogenesis, thus increasing the blood level of glucose. *Epinephrine* is produced by the adrenal medulla. It promotes glycogenolysis, thus increasing blood glucose. *Growth hormone* and *adrenocorticotropic hormone* are produced by the anterior pituitary gland. Both hormones are antagonistic to insulin and hence increase blood glucose. *Thyroxine* is produced by the thyroid gland. It not only stimulates glycogenolysis but also increases the intestinal absorption rate of glucose.

172.

B. Epinephrine is produced by the adrenal medulla. It promotes glycogenolysis, thus increasing the blood glucose level. Epinephrine also inhibits the secretion of insulin and stimulates the secretion of glucagon.

173.

A. In Cushing syndrome the adrenal cortex secretes an excessive amount of the hormone cortisol. Because cortisol has a stimulatory effect on gluconeogenesis, hyperglycemia commonly occurs as a secondary disorder. Hypoglycemia frequently characterizes Addison disease in which there is decreased production of cortisol.

174.

B. When a fasting plasma glucose test is performed and the glucose value is between 100–125 mg/dL, the individual is considered to have impaired fasting glucose (IFG). This is less than the value associated with diagnosis of diabetes mellitus, which is a fasting plasma glucose $\geq$126 mg/dL. IFG is considered a risk factor and a stage between normal glucose metabolism and development of diabetes mellitus.

175.

B. Because of the critical reasons for aspirating a CSF specimen, the testing is performed as soon as possible upon receipt of the specimen in the laboratory. In this case, the cloudy appearance would be most likely due to the presence of bacteria. Both bacteria and red blood cells can use glucose *in vitro*. Thus any delay in glucose testing could result in a falsely low result. The CSF specimen should be centrifuged to remove cellular material and assayed immediately.

176.

A. In the glucose oxidase/peroxidase method, the second coupled enzyme reaction involves peroxidase catalyzing the reaction between hydrogen peroxide and a chromogenic oxygen acceptor, which is oxidized to its colored form. Several blood constituents, including uric acid, ascorbic acid, bilirubin, tetracycline, hemoglobin, and glutathione, when present in increased concentrations can interfere with the assay by competing for the hydrogen peroxide produced in the first coupled enzyme reaction. This loss of hydrogen peroxide would result in falsely low plasma glucose results. Because of the high levels of uric acid normally found in urine, the glucose oxidase/peroxidase method would not be suitable for measuring urine glucose.

177.

D. A casual plasma glucose should be less than 200 mg/dL. The reference range for glycated hemoglobin (Hb A_{1c}) is 4–6%. Because the individual is a postmenopausal, 57-year-old female, with abnormal test results being found as part of an annual physical examination, the most likely diagnosis is type 2 diabetes mellitus. The ADA recommends that in the absence of unequivocal hyperglycemia, the glucose result should be confirmed by repeating the casual glucose or performing a fasting plasma glucose on a subsequent day. The ADA does not recommend Hb A_{1c} as a screening test for diabetes mellitus.

178.

A. Carbohydrate is stored in the body in the form of glycogen. There are many enzymes involved in the metabolism of glycogen. A deficiency of any one of the enzymes involved will result in what are called glycogen storage diseases, or glycogenoses. There are at least 10 distinct types of glycogen storage diseases, and all of them are rare. All are hereditary. Diagnosis of each type can be made by the assay of the deficient enzyme from the appropriate tissue and by microscopic study of the affected tissues.

- Type I—von Gierke disease is clinically characterized by severe fasting hypoglycemia and lactic acidosis. This is due to a deficiency of the enzyme glucose-6-phosphatase. Glucose cannot be transported from the liver as glucose-6-phosphate during the breakdown of glycogen. It is metabolized to lactic acid and thus results in lactic acidosis.
- Type II—Pompe disease is caused by a deficiency of lysosomal α-1,4-glucosidase. This results in an increase of glycogen in all organs and abnormally large lysosomes. The glycogen cannot be degraded because of the deficiency of α-1,4-glucosidase.
- Type III—Cori disease is caused by the absence of a debrancher enzyme. This disease is characterized by hypoglycemia, hepatomegaly, seizures, and growth retardation.
- Type IV—Andersen disease is caused by a deficiency of brancher enzyme. It is a rare disease characterized by progressive liver enlargement or cirrhosis and muscular weakness by the age of 2 months. Storage glycogen is not usually found, but unbranched amylopectin accumulates in this disease.

Lipids and Lipoproteins

179.

C. Bile acids are synthesized in the hepatocytes of the liver. They are C_{24} steroids that are derived from cholesterol. With fat ingestion, the bile salts are released into the intestines, where they aid in the emulsification of dietary fats. Thus bile acids also serve as a vehicle for cholesterol excretion. A majority of the bile acids, however, are reabsorbed from the intestines into the enterohepatic circulation for reexcretion into the bile. The two principal bile acids are cholic acid and chenodeoxycholic acid. These acids are conjugated with one of two amino acids, glycine or taurine. Measurement of bile acids is possible via immunotechniques and may aid in the diagnosis of some liver disorders such as obstructive jaundice, primary biliary cirrhosis, and viral hepatitis.

180.

C. After fat ingestion, lipids are first degraded, then reformed, and finally incorporated by the intestinal mucosal cells into absorbable complexes known as chylomicrons. These chylomicrons enter the blood through the lymphatic system, where they impart a turbid appearance to serum. Such lipemic plasma specimens frequently interfere with absorbance or cause a change in absorbance measurements, leading to invalid results.

181.

C. Total cholesterol consists of two fractions, free cholesterol and cholesteryl ester. In the plasma, cholesterol exists mostly in the cholesteryl ester form. Approximately 70% of total plasma cholesterol is esterfied with fatty acids. The formation of cholesteryl esters is such that a transferase enzyme catalyzes the transfer of fatty acids from phosphatidylcholine to the carbon-3 alcohol function position of the free cholesterol molecule. Laboratories routinely measure total cholesterol by first using the reagent cholesterol esterase to break the ester bonds with the fatty acids.

182.

D. A "routine" lipid profile would most likely consist of the measurement of total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol. These measurements are most easily adapted to today's multichannel chemistry analyzers. Both total cholesterol and triglyceride use enzymatic techniques to drive the reaction to completion. HDL cholesterol and LDL cholesterol are commonly requested tests to help determine patient risk for coronary heart disease. The HDL is separated from other lipoproteins using a precipitation technique, immunotechniques, and/or polymers and detergents. The nonprecipitation techniques are preferred because they can give better precision, be adapted to an automated chemistry analyzer, and be run without personnel intervention. LDL cholesterol may be calculated using the Friedewald equation, or it may be assayed directly using selective precipitation methods or direct homogeneous techniques.

183.

D. Blood specimens for lipid studies should be drawn in the fasting state at least 9 to 12 hours after eating. Although fat ingestion only slightly affects cholesterol levels, the triglyceride results are greatly affected. Triglycerides peak at about 4 to 6 hours after a meal, and these exogenous lipids should be cleared from the plasma before analysis. The presence of chylomicrons, as a result of an inadequate fasting period, must be avoided because of their interference in spectrophotometric analyses.

184.

A. Total cholesterol screenings are commonly performed on nonfasting individuals. Total cholesterol is only slightly affected by the fasting status of the individual, whereas triglycerides, fatty acids, and lipoproteins are greatly affected. Following a meal, chylomicrons would be present, which are rich in triglycerides and fatty acids and contain very little cholesterol. The majority of cholesterol is produced by the liver and other tissues. High levels of exogenous triglycerides and/or fatty acids will interfere with the measurement of lipoproteins. Chylomicrons are normally cleared from the body 6 hours after eating.

185.

B. The long-chain fatty acids of triglycerides can be broken down to form energy through the process of beta/oxidation, also known as the fatty acid cycle. In this process, two carbons at a time are cleaved from long-chain fatty acids to form acetyl-coenzyme A. Acetyl-coenzyme A, in turn, can enter the Krebs cycle to be converted to energy or be converted to acetoacetyl-Co-A and converted to energy by an alternate pathway, leaving behind the acidic by-product ketones composed of beta-hydroxybutyrate, acetoacetate, and acetone. Under proper conditions, pyruvate can be converted to acetyl-coenzyme A at the end of glycolysis of glucose. Bile is a breakdown product of cholesterol used in the digestion of dietary cholesterol.

186.

D. The kinetic methods used for quantifying serum triglycerides use a reaction system of coupling enzymes. It is first necessary to hydrolyze the triglycerides to free fatty acids and glycerol. This hydrolysis step is catalyzed by the enzyme lipase. The glycerol is then free to react in the enzyme-coupled reaction system that includes glycerokinase, pyruvate kinase, and lactate dehydrogenase or in the enzyme-coupled system that includes glycerokinase, glycerophosphate oxidase, and peroxidase.

187.

C. In the enzymatic method for quantifying total cholesterol in serum, the serum specimen must initially be treated with cholesteryl ester hydrolase. This enzyme hydrolyzes the cholesteryl esters into free cholesterol and fatty acids. Both the free cholesterol, derived from the cholesteryl ester fraction, and any free cholesterol normally present in serum may react in the cholesterol oxidase/peroxidase reactions for total cholesterol. The hydrolysis of the cholesteryl ester fraction is necessary because cholesterol oxidase reacts only with free cholesterol.

188.

C. Chylomicrons are protein-lipid complexes composed primarily of triglycerides and containing only small amounts of cholesterol, phospholipids, and protein. After food ingestion, the chylomicron complexes are formed in the epithelial cells of the intestines. From the epithelial cells, the chylomicrons are released into the lymphatic system, which transports chylomicrons to the blood. The chylomicrons may then carry the triglycerides to adipose tissue for storage, to organs for catabolism, or to the liver for incorporation of the triglycerides into very-low-density lipoproteins (VLDLs). Chylomicrons are normally cleared from plasma within 6 hours after a meal.

189.

B. Beta-hydroxybutyric acid, acetoacetic acid, and acetone are collectively referred to as ketone bodies. They are formed as a result of the process of beta-oxidation in which liver cells degrade fatty acids with a resultant excess accumulation of acetyl-coenzyme A (CoA). The acetyl-CoA is the parent compound from which ketone bodies are synthesized through a series of reactions.

190.

D. Sphingolipids, most notably sphingomyelin, are the major lipids of the cell membranes of the central nervous system (i.e., the myelin sheath). Like phospholipids, sphingolipids are amphipathic and contain a polar, hydrophilic head and a nonpolar, hydrophobic tail, making them excellent membrane formers. Although sometimes considered a subgroup of phospholipids, sphingomyelin is derived from the amino alcohol sphingosine instead of glycerol.

191.

B. All the lipoproteins contain some amount of triglyceride, cholesterol, phospholipid, and protein. Each of the lipoprotein fractions is distinguished by its unique concentration of these substances. The beta-lipoprotein fraction is composed of approximately 50% cholesterol, 6% triglycerides, 22% phospholipids, and 22% protein. The beta-lipoproteins, which are also known as the low-density lipoproteins (LDLs), are the principal transport vehicle for cholesterol in the plasma. Both the chylomicrons and the prebeta-lipoproteins are composed primarily of triglycerides. The chylomicrons are considered transport vehicles for exogenous triglycerides. In other words, dietary fat is absorbed through the intestine in the form of chylomicrons. After a meal, the liver will clear the chylomicrons from the blood and use the triglyceride component to form the prebeta-lipoproteins. Therefore, in the fasting state triglycerides are transported in the blood primarily by the prebeta-lipoproteins. The prebeta-lipoproteins are composed of approximately 55% triglycerides.

192.

B. The 27-carbon, ringed structure of cholesterol is the backbone of steroid hormones. The nucleus is called the cyclopentanoperhydrophenanthrene ring. The steroid hormones having this ring include estrogens (18 carbons), androgens (19 carbons), glucocorticoids (21 carbons), and mineralocorticoids (21 carbons).

193.

C. The majority of the lipid (lysosomal) storage diseases are inherited as autosomal recessive mutations. This group of diseases is characterized by an accumulation of sphingolipids in the central nervous system or some other organ. Such lipid accumulation frequently leads to mental retardation or progressive loss of central nervous system functions. The cause of such lipid accumulation has been attributed either to specific enzyme deficiencies or to nonfunctional enzyme forms that inhibit the normal catabolism of the sphingolipids.

194.

B. Pancreatic insufficiency, Whipple disease, cystic fibrosis, and tropical sprue are diseases characterized by the malabsorption of lipids from the intestines. This malabsorption results in an excess lipid accumulation in the feces that is known as steatorrhea. When steatorrhea is suspected, the amount of lipid material present in the feces may be quantified. A 24- or 72-hour fecal specimen should be collected, the latter being the specimen of choice. The lipids are extracted from the fecal specimen and analyzed by gravimetric or titrimetric methods.

195.

B. A double nomenclature exists for the five principal lipoprotein fractions. The nomenclature is such that the various fractions have been named on the basis of both the electrophoretic mobilities and the ultracentrifugal sedimentation rates. The chylomicrons are known as chylomicrons by both methods. The chylomicrons are the least dense fraction, exhibiting a solvent density for isolation of less than 0.95 g/mL, and have the slowest electrophoretic mobility. The HDLs, also known as the alpha-lipoproteins, have the greatest density of 1.063–1.210 g/mL and move the fastest electrophoretically toward the anode. The VLDLs, also known as the prebeta-lipoproteins, move slightly slower electrophoretically than the alpha fraction. The VLDLs have a density of 0.95–1.006 g/mL. The IDLs, intermediate-density lipoproteins, have a density of 1.006–1.019 g/mL and migrate as a broad band between beta- and prebeta-lipoproteins. The LDLs, also known as the beta-lipoproteins, have an electrophoretic mobility that is slightly slower than that of the IDL fraction. The LDLs have an intermediate density of 1.019–1.063 g/mL, which is between the IDLs and the HDLs. To summarize the electrophoretic mobilities, the alpha-lipoprotein fraction migrates the farthest toward the anode from the origin, followed in order of decreasing mobility by the prebeta-lipoprotein, broad band between beta- and prebeta-lipoprotein, beta-lipoprotein, and chylomicron fractions. The chylomicrons remain more cathodic near the point of serum application.

196.

C. The quantification of the HDL cholesterol level is thought to contribute in assessing the risk that an individual may develop coronary artery disease (CAD). There appears to be an inverse relationship between HDL cholesterol and CAD. With low levels of HDL cholesterol, the risk of CAD increases. It is thought that the HDL facilitates the removal of cholesterol from the arterial wall, therefore decreasing the risk of atherosclerosis. In addition, LDL cholesterol may be assessed, because increased LDL cholesterol and decreased HDL cholesterol are associated with increased risk of CAD.

197.

D. Respiratory distress syndrome (RDS), also referred to as hyaline membrane disease, is commonly seen in preterm infants. A deficiency of pulmonary surfactant causes the infant's alveoli to collapse during expiration, resulting in improper oxygenation of capillary blood in the alveoli. Currently, the surfactant/albumin ratio by fluorescence polarization is performed using amniotic fluid to assess fetal lung maturity. The amniotic fluid is mixed with a fluorescent dye. When the dye binds to albumin there is a high polarization, and when the dye binds to surfactant there is a low polarization. Thus the surfactant/albumin ratio is determined. The units are expressed as milligrams of surfactant per gram of albumin, with fetal lung maturity being sufficient with values greater than 50 mg/g. Older methodologies have employed the determinations of phosphatidylglycerol, foam stability, and lecithin/sphingomyelin (L/S) ratio. The L/S ratio is based on the physiological levels of lecithin and sphingomyelin. Lecithin is a surfactant that prepares lungs to expand and take in air. Sphingomyelin is incorporated into the myelin sheath of the central nervous system of the fetus. The amounts of lecithin and sphingomyelin produced during the first 34 weeks of gestation are approximately equal; however, after the 34th week, the amount of lecithin synthesized greatly exceeds that of sphingomyelin. At birth, an L/S ratio of 2:1 or greater would indicate sufficient lung maturity.

198.

C. The VLDL fraction is primarily composed of triglycerides and lesser amounts of cholesterol and phospholipids. Protein components of VLDL are mostly apolipoprotein B-100 and apolipoprotein C. VLDL migrates electrophoretically in the prebeta region.

199.

A. The patient is a known diabetic who has been experiencing chest pain and shortness of breath with activity. The ECG was normal. The most likely diagnosis is angina pectoris. The LDL cholesterol result does not correlate with the other lipid results, and it appears to be less than what would be expected. Using the formula LDL cholesterol = total cholesterol – [HDL cholesterol + triglycerides/5], the calculated LDL cholesterol would be 192 mg/dL. The total cholesterol, HDL cholesterol, and triglyceride results correlate and indicate hyperlipidemia. The elevated fasting glucose indicates poor carbohydrate metabolism, and the elevated hemoglobin A_{1c} indicates a lack of glucose control during the previous 2 to 3 months. The elevated glucose and lipid results support an increased risk of coronary artery disease, as does the hs-CRP value, which falls in the high risk range (>3.0 mg/L).

200.

C. Either a dextran sulfate-magnesium chloride mixture or a heparin sulfate-manganese chloride mixture may be used to precipitate the LDL and VLDL cholesterol fractions. This allows the HDL cholesterol fraction to remain in the supernatant. An aliquot of the supernatant may then be used in a total cholesterol procedure for the quantification of the HDL cholesterol level.

201.

B. Both the direct and the heparin sulfate-manganese chloride precipitation methods measure HDL cholesterol. The direct or homogeneous method for HDL cholesterol uses a mixture of polyanions and polymers that bind to LDL and VLDL and chylomicrons, causing them to become stabilized. The polyanions neutralize ionic charges on the surface of the lipoproteins, and this enhances their binding to the polymer. When a detergent is added, HDL goes into solution, whereas the other lipoproteins remain attached to the polymer/polyanion complexes. The HDL cholesterol then reacts with added cholesterol enzyme reagents while the other lipoproteins remain inactive. The reagents, polymer/polyanions, and detergent can be added to the specimen in an automated way without the need for any manual pretreatment step. Furthermore, the direct HDL cholesterol procedure has the capacity for better precision than the manual precipitation methods. Both the adaptability to automated instruments and the better precision make the direct method a preferred choice for quantifying HDL cholesterol.

202.

A. A number of risk factors are associated with developing coronary heart disease. Notable among these factors are increased total cholesterol and decreased HDL cholesterol levels. Although the reference ranges for total cholesterol and HDL cholesterol vary with age and sex, reasonable generalizations can be made: An HDL cholesterol less than 40 mg/dL and a total cholesterol value ≥240 mg/dL are undesirable and the individual is at greater risk for coronary heart disease. Total cholesterol values between 200 and 239 mg/dL are borderline high.

203.

B. Once the total cholesterol, triglyceride, and HDL cholesterol are known, LDL cholesterol can be quantified by using the Friedewald equation

$$\text{LDL cholesterol} = \text{Total cholesterol} - (\text{HDL cholesterol} + \text{Triglyceride}/5)$$

In this example, all results are in mg/dL:

$$\begin{aligned}\text{LDL cholesterol} &= 300 - (50 + 200/5)\\ &= 300 - (90)\\ &= 210\ \text{mg/dL}\end{aligned}$$

This estimation of LDL cholesterol has been widely accepted in routine clinical laboratories and can be easily programmed into laboratory computers. In addition, LDL methods are available for direct measurement of serum levels. *Note:* The equation should not be used with triglyceride values exceeding 400 mg/dL because the VLDL composition is abnormal, making the [triglyceride/5] factor inapplicable.

204.

D. Both total cholesterol and HDL cholesterol are independent measurable indicators of risk of coronary heart disease (CHD). By relating total and HDL cholesterol in a mathematical way, physicians can obtain valuable additional information in predicting risk for CHD. Risk of CHD can be quantified by the ratio of total cholesterol to HDL cholesterol along the following lines:

Ratio	Risk CHD
3.43	half average
4.97	average
9.55	two times average
24.39	three times average

Thus this patient shows approximately twice the average risk for CHD. Risk ratios for CHD can easily be calculated by instrument and/or laboratory computers given the total and HDL cholesterol values. Reports indicating level of risk based on these results can be programmed by the laboratory and/or manufacturer.

205.

C. A number of immunochemical assays can be used to quantify the apolipoproteins. Some of the techniques that can be used include immunonephelometric assay, enzyme-linked immunosorbent assay (ELISA), and immunoturbidimetric assay. Commercial kits are available for the quantification of Apo A-I and Apo B-100. Measuring the apolipoproteins can be of use in assessing increased risk for coronary heart disease.

206.

A. Lipoprotein (a) is an apolipoprotein that is more commonly referred to as Lp(a). Although it is related structurally to LDL, Lp(a) is considered to be a distinct lipoprotein class with an electrophoretic mobility in the prebeta region. Lp(a) is believed to interfere with the lysis of clots by competing with plasminogen in the coagulation cascade, thus increasing the likelihood of atherosclerotic cardiovascular disease.

207.

B. Historically, total cholesterol levels of Americans have been below 300 mg/dL. Other countries, however, have relatively lower population cholesterol levels. The prevalent diet of these countries, however, may be vegetarian or fish, as opposed to meat, oriented. Higher total cholesterol resulting from a meat diet has been established. Clinical studies have also shown an increased risk of CAD in individuals with total cholesterol greater than 200 mg/dL. Thus, the upper reference interval of acceptable total cholesterol was artificially lowered to 200 mg/dL to reflect the lower risk of CAD associated with it.

208.

D. The Abell-Kendall assay is commonly used to separate HDL cholesterol from other lipoproteins. In this precipitation technique a heparin sulfate-manganese chloride mixture is used to precipitate the LDL and VLDL cholesterol fractions. This technique works well as long as there is no significant amount of chylomicrons or lipemia in the specimen and/or the triglyceride is under 400 mg/dL. Incomplete sedimentation is seen as cloudiness or turbidity in the *supernatant* after centrifugation. It indicates the presence of other lipoproteins and leads to over estimation of HDL cholesterol. The lipemic specimens may be cleared and the HDL cholesterol separated more effectively by using ultrafiltration, extraction, latex immobilized antibodies, and/or ultracentrifugation. These techniques are usually not available in a routine laboratory.

209.

A. Hyperlipoproteinemia can be genetically inherited or secondary to certain diseases such as diabetes mellitus, hypothyroidism, or alcoholism. If the alcoholism has advanced to the state where there is liver damage, the liver can become inefficient in its metabolism of fats, leading to an increase of total cholesterol, triglyceride, LDL, and/or VLDL in the bloodstream. The elevation of these lipids along with the previous liver damage (e.g., cirrhosis) leads to a poor prognosis for the patient.

210.

D. In evaluating lipid profile results, it is important to start with the integrity of the sample. From the case history, it is doubtful that a 10-year-old healthy, active boy would be suffering from a lipid or glucose disorder manifesting these kinds of results. Furthermore, the boy came in for testing after school. It is improbable that a 10-year-old boy would be able to maintain a 9- to 12-hour fast during the school day. In this case, the boy should have been thoroughly interviewed by the laboratory staff before the blood test to determine if he was truly fasting. Specimen integrity is the first thing that must be ensured before running any glucose or lipid tests.

211.

C. In this case, the child fits the description of a suspected hyperlipemic patient. He is known to have diabetes mellitus, and the mother has assured the laboratory that the boy has followed the proper fasting protocol before the test. Hyperlipoproteinemia can be secondary to diabetes mellitus. The boy has a relatively high risk to develop CAD, and, as a known diabetic, should never undergo an oral 3-hour glucose tolerance test.

Enzymes and Cardiac Assessment

212.

C. The majority of serum enzymes that are of interest clinically are of intracellular origin. These enzymes function intracellularly, with only small amounts found in serum as a result of normal cellular turnover. Increased serum levels are due to tissue damage and necrosis, where the cells disintegrate and leak their contents into the blood. Thus, elevated serum levels of intracellular enzymes are used diagnostically to assess tissue damage.

213.

B. Enzymes are proteins that act as catalysts. It is not practical to measure enzyme concentrations in a body fluid specimen, but rather to assay enzymes according to their activity in catalyzing an appropriate reaction; that is, the conversion of substrate to product. An enzyme acts by combining with a specific substrate to form an enzyme-substrate complex, which then breaks down into product plus free enzyme, which is reused. A general form of the reaction is

$$[E] + [S] \rightleftarrows [ES] \rightarrow [P] + [E]$$

where [E] = concentration of enzyme, [S] = concentration of substrate, [ES] = concentration of enzyme-substrate complex, and [P] = concentration of product of the reaction. Because the rate of such a reaction is used as a measure of enzyme activity, it is important to consider the effect of substrate concentration on the rate of the reaction. The kinetics of the reaction are initially of the first order (i.e., the rate varies with the concentration of substrate as well as the concentration of enzyme) until there is sufficient substrate present to combine with all enzyme. The reaction rate then becomes zero order (i.e., the rate is independent of concentration of substrate and directly proportional to concentration of enzyme as measured by reaction rate) when substrate is present in excess. Hence it is desirable to use conditions that provide zero-order kinetics when assaying enzyme activity.

214.

B. Michaelis and Menten proposed a basis for the theory of enzyme-substrate complexes and rate reactions. By measuring the velocity of the reaction at varying substrate concentrations, it is possible to determine the Michaelis constant (K_m) for any specific enzymatic reaction. K_m represents the specific concentration of substrate that is required for a particular reaction to proceed at a velocity that is equal to half of its maximum velocity. The K_m value tells something about the affinity of an enzyme for its substrate. When [S] = K_m, the velocity of the reaction is expressed as $V = 1/2\ V_{max}$. In the graph shown with this question, the K_m of the reaction is represented by *b*. Because substrate must be present in excess to obtain zero-order kinetics, the substrate concentration necessary would have to be at least 10 times the K_m, which is represented by *d*. Usually substrate concentrations 20–100 times the K_m are used to be sure that substrate is present in excess. Thus it is critical that the K_m value be determined experimentally.

215.

A. Factors that affect enzyme assays include temperature, pH, substrate concentration, and time of incubation. For each clinically important enzyme, the optimum temperature and pH for its specific reaction are known. When lower than optimum temperature or pH is employed, the measured enzyme activity will be lower than the expected activity value. As temperature increases, the rate of the reaction increases. Generally, a twofold increase in reaction rates will be observed with a 10°C rise in temperature. However, once the optimum temperature is exceeded, the reaction rate falls off as enzyme denaturation occurs at temperatures ranging from 40 to 70°C.

216.

D. An international unit (IU) is defined as the enzyme activity that catalyzes the conversion of 1 μmol of substrate in 1 minute under standard conditions. For determination of enzyme activity when a rate method is employed, the following equation is used:

$$\frac{\Delta A/\text{min} \times \text{volume (mL)} \times 10^6\,\mu\text{mol/mol}}{\text{Absorptivity} \times \text{light path} \times \text{specimen volume (mL)}} = \frac{\text{total assay}}{\text{coefficient (cm)}} = \text{IU/L}$$

$$\frac{0.077 \times 3.02\ \text{mL} \times 10^6\,\mu\text{mol/mol}}{6.22 \times 10^3\,\text{L/mol} \cdot \text{cm} \times 1\ \text{cm} \times 0.02\ \text{mL}} = 1869\ \text{IU/L}$$

It is important to remember that the total assay volume includes the volume of reagent, diluent, and sample used in the particular assay and that the total assay volume and specimen volume should be expressed in the same units.

217.

B. Enzymes are protein in nature. Like all proteins, they may be denatured with a loss of activity as a result of several factors (e.g., heat, extreme pH, mechanical agitation, strong acids, and organic solvents). Enzymes act as catalysts for the many chemical reactions of the body. Enzymes increase the rate of a specific chemical reaction by lowering the activation energy needed for the reaction to proceed. They do not change the equilibrium constant of the reaction; but rather, enzymes affect the rate at which equilibrium occurs between reactants and products.

218.

C. Serum alkaline phosphatase is elevated in several disorders, including hepatobiliary and bone diseases. For an accurate assay of most serum enzymes, the presence of hemolyzed red blood cells must be avoided because many enzymes are present in red cells. Serum aspartate transaminase (formerly known as glutamate-oxaloacetate transaminase, GOT) and lactate dehydrogenase are both enzymes that are elevated in acute myocardial infarction and liver disease.

219.

D. There are six major classes of enzymes. The International Commission of Enzymes of the International Union of Biochemistry has categorized all enzymes into one of these classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Transferases are enzymes that catalyze the transfer of groups, such as amino and phosphate groups, between compounds. Transferases frequently need coenzymes, such as pyridoxal-5′-phosphate (P-5′-P), for the amino transfer reactions. Aspartate and alanine aminotransferases, creatine kinase, and gamma-glutamyltransferase are typical examples.

220.

B. Hydrolases are enzymes that split molecules with the addition of water—for example, amylase, lipase, alkaline phosphatase, acid phosphatase, 5′-nucleotidase, and trypsin. They do not usually require coenzymes but often need activators. Aldolase and carbonic anhydrase are examples of the class of enzymes known as the lyases. Lyases are enzymes that split molecules between carbon-to-carbon bonds without the addition of water. The resulting products usually contain carbon double bonds.

221.

C. The oxidoreductases are enzymes that catalyze the addition or removal of hydrogen from compounds. These enzymes need a coenzyme, such as nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated derivative $NADP^+$, as a hydrogen acceptor or donor in order to function. Lactate dehydrogenase and glucose-6-phosphate dehydrogenase are examples of oxidoreductases. Isomerases are those enzymes that catalyze intramolecular conversions such as the oxidation of a functional group by an adjacent group within the same molecule. Glucose phosphate isomerase is an example of this class of enzymes. Ligases are those enzymes that catalyze the union of two molecules accompanied by the breakdown of a phosphate bond in adenosine triphosphate (ATP) or a similar triphosphate. An example is glutamine synthetase.

222.

B. Aspartate and alanine aminotransferases catalyze the transfer of amino groups between amino acids and α-oxoacids. A prosthetic group, pyridoxal-5′-phosphate (P-5′-P), is required for the transfer of the amino group. In the aspartate aminotransferase (AST) reaction, AST catalyzes the transfer of an amino group from L-aspartate to α-oxoglutarate, with the amino group transfer mediated by P-5′-P, which is bound to the apoenzyme. The products formed are oxaloacetate and L-glutamate. By coupling this reaction with a malate dehydrogenase reaction, the decrease in absorbance of NADH as it is oxidized to NAD^+ can be followed at 340 nm. The change in absorbance will be proportional to the AST activity present in the serum specimen.

223.

D. Alanine aminotransferase (ALT), formerly known as glutamate pyruvate transaminase (GPT), and aspartate aminotransferase (AST), formerly known as glutamate oxaloacetate transaminase (GOT), are categorized as transferase enzymes. These older designations are still seen in conjunction with the current terminology on reagent packaging, on physician test request forms, and on laboratory test result forms. Through the transfer of amino groups, ALT and AST catalyze the interconversion of amino acids and keto acids. ALT catalyzes the interconversion of alanine and oxoglutarate to pyruvate and glutamate. The reaction is reversible. In viral hepatitis, both ALT and AST are elevated. In acute myocardial infarction, AST is elevated and ALT is normal or slightly increased.

$$\text{L-Alanine} + \alpha\text{-oxoglutarate} \xrightleftharpoons[\text{P-5'-P}]{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

224.

D. When measuring CK-MB, the mass immunoassay is more sensitive because it is quantifying the amount of enzyme present. This is in contrast to a kinetic method, which measures enzyme activity by means of the enzyme catalyzing a reaction and the product of that reaction being measured. Electrophoretic methods also measure enzyme activity based on colored product or fluorescent product formation.

225.

A. Lactate dehydrogenase (LD, also abbreviated LDH) is found in all body tissues and is especially abundant in red and white blood cells. Hence hemolyzed serum will give falsely elevated results for LD. The enzyme catalyzes the conversion of lactate to pyruvate at pH 8.8–9.8 and pyruvate to lactate at pH 7.4–7.8, mediated by nicotinamide adenine dinucleotide (NAD^+). Each of these reactions is associated with its own unique reference range. LD exists in five isomeric forms called isoenzymes. The isoenzymes can be separated by electrophoresis. Serum specimens for LD isoenzyme determinations can be stored at room temperature for 2 or 3 days without appreciable loss of activity. Room temperature storage is necessary because LD-4 and LD-5 are labile in the cold. This is in contrast to most enzymes, which are more stable when refrigerated or frozen.

226.

C. C-reactive protein is an acute-phase reactant that is increased in the presence of inflammation. High-sensitivity C-reactive protein (hs-CRP) refers to a sensitive method that is able to measure low levels of CRP in serum. One theory is that elevated levels of CRP contribute to the damage of arterial walls that precedes plaque formation. hs-CRP is considered a good predictor test for assessing cardiovascular risk. However, it is also elevated in other conditions, including infection, stress, and trauma. CK-MB, troponin, and myoglobin are tests used to assess if a myocardial infarction has occurred.

227.

C. Enzymes catalyze specific reactions or closely related groups of reactions. Lactate dehydrogenase (LD), with nicotinamide adenine dinucleotide (NAD^+) as a hydrogen acceptor, catalyzes the oxidation of L-lactate to pyruvate and the reduction of NAD^+ to NADH. Because NAD^+ does not absorb light at 340 nm but NADH does, the production of NADH can be monitored as an increase in absorbance at 340 nm and related to the LD activity present in the specimen. Because this reaction is reversible, either the forward or reverse reaction can be used in the laboratory to quantify LD activity. Although the reaction equilibrium favors the formation of lactate from pyruvate, this reaction is less commonly used. It should be noted that the reference ranges for the two reactions are considerably different. Elevation of serum LD is associated with acute myocardial infarction, liver disease, pernicious anemia, malignant disease, and pulmonary embolism. It is also seen in some cases of renal disease, especially where tubular necrosis or pyelonephritis exists.

228.

B. In acute myocardial infarction (AMI), the initial increase in serum myoglobin levels occurs in 1 to 3 hours following onset of symptoms. Serial measurements need to be made because a single value is not diagnostic. When doubling of the initial value occurs within 1 to 2 hours, this is suggestive of AMI. In AMI, the myoglobin level will peak within 5 to 12 hours, with serum levels returning to normal within 18 to 30 hours. Because myoglobin is found in other tissues and is not cardiac specific, it is usually used in conjunction with cardiac troponin and CK-MB to assess the occurrence of AMI.

229.

C. Increased serum creatine kinase (CK), formerly called creatine phosphokinase (CPK), values are caused primarily by lesions of cardiac muscle, skeletal muscle, or brain tissue. CK increases in the early stages of Duchenne-type progressive muscular dystrophy. Assays of total CK and CK isoenzymes are commonly used in the diagnosis of myocardial infarction. Hypothyroidism causes a moderate increase in CK values. Elevation of this enzyme also occurs after vigorous muscular activity, in cases of cerebrovascular accidents (stroke), and after repeated intramuscular injections. In addition to quantifying total CK activity, isoenzymes may be determined by using electrophoretic, immunologic, or ion-exchange chromatography methods. Three isoenzymes have been identified: CK-1 or BB, primarily found in brain and nerve tissues with some in thyroid, kidney, and intestine; CK-2 or MB, primarily found in heart muscle; and CK-3 or MM, primarily found in skeletal muscle but present in all body tissues. CK is not elevated in bone disease.

230.

C. Creatine kinase (CK) is found mainly in skeletal muscle, cardiac muscle, and brain tissue. CK catalyzes the following reversible reaction:

$$\text{creatine} + \text{adenosine triphosphate (ATP)} \underset{\text{pH 6.7}}{\overset{\text{pH 9.0}}{\rightleftharpoons}} \text{phosphocreatine} + \text{adenosine diphosphate (ADP)}$$

Mg^{2+} is required as an activator. The direction in which the reaction takes place, and hence the equilibrium point, depends on the pH. Measurement of CK activity is valuable in the early diagnosis of acute myocardial infarction. Its level rises 4 to 6 hours after infarction, reaches its peak at 12 to 24 hours, and returns to normal by the third day. In addition to quantifying total CK activity, electrophoresis may be performed to ascertain the presence of an MB band, which represents the heart tissue isoenzyme. Electrophoretically, the MB band moves to an intermediary position between the BB and the MM bands. The BB band travels fastest toward the anode and the MM band travels slowest, remaining in the gamma-globulin region. Electrophoretic separation of CK-MB has been widely replaced by immunologic methods that can be performed on automated instruments.

231.

B. The function of amylase to catalyze the hydrolysis of starch to dextrins, maltose, and glucose has been used as the basis for several methods over the years. The more commonly used methods today employ small oligosaccharides and 4-nitrophenyl-glycoside as substrates. In general, these methods can be automated, using an oxygen electrode system and UV or visible wavelength spectrophotometry to determine amylase activity.

232.

D. Gamma-glutamyltransferase (GGT) catalyzes the transfer of gamma-glutamyl groups from peptides to an appropriate acceptor. GGT is found in almost all cells. The highest amount of GGT is found in the kidney, and slightly less is found in the liver and pancreas. Diagnostically, the assay of GGT is widely used to investigate hepatic disease. Increased values are seen in a variety of liver disorders and in conditions that are characterized by secondary liver involvement, including acute pancreatitis, pancreatic carcinoma, infectious mononucleosis, alcoholism, and cardiac insufficiency. Normal GGT levels are seen in bone disorders, in growing children, and during pregnancy.

233.

C. The main sources of alkaline phosphatase are liver, bone, intestine, and placenta. Elevated serum alkaline phosphatase is associated with liver disease and with both obstructive jaundice and intrahepatic jaundice. In most cases, the serum alkaline phosphatase value in obstructive jaundice is higher than in intrahepatic jaundice. Increased serum values are also found in bone diseases such as Paget disease; in pregnant women (placental origin), especially in the third trimester of a normal pregnancy; and in normal growing children. In the presence of the latter conditions, when liver disease is also suspected, a GGT assay may be performed to aid in a differential diagnosis. Serum GGT levels are normal in these conditions but are elevated in liver disease.

234.

B. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, and lactate dehydrogenase are enzymes for which the serum activities may be assayed to assess liver function. At the cellular level, alkaline phosphatase functions in the membrane border, gamma-glutamyltransferase functions in the cell membrane, and alanine aminotransferase functions both in the cytoplasm and mitochondria. With tissue damage and necrosis, the cells disintegrate and leak their contents into the blood. Because these enzymes are cellular enzymes, any increase in their activity levels in serum is indicative of tissue destruction. It is important to remember that these enzyme levels must be used in conjunction with other clinical data because enzymes generally are not organ specific; they are found in several tissues.

235.

B. Amylase and lipase are the two most important enzymes in evaluating pancreatic function. The values of amylase and lipase activity are significantly elevated in acute pancreatitis and obstruction of the pancreatic duct. In most cases of acute pancreatitis, the lipase activity stays elevated longer than amylase activity.

236.

B. The quantification of serum prostate-specific antigen has replaced measurement of serum acid phosphatase for assessing carcinoma of the prostate. PSA measurement in conjunction with the digital rectal examination is recommended for prostate cancer screening. In addition, PSA can be used to stage and monitor therapy of prostatic cancer.

237.

B. Cholinesterase is a serum enzyme synthesized by the liver. It is also known as pseudocholinesterase to distinguish it from "true" cholinesterase (acetylcholinesterase) of erythrocytes. Although a number of disease states are associated with abnormal levels of this enzyme, cholinesterase levels are especially important in detecting organic insecticide poisoning of workers in the chemical industry and agriculture. Decreased cholinesterase levels and atypical enzyme forms are associated with prolonged apnea after succinylcholine administration during surgery. Propionylthiocholine is a commonly used substrate for measuring serum cholinesterase activity.

238.

D. The heme protein myoglobin can bind oxygen reversibly and is found in cardiac and striated muscles. In cases of acute myocardial infarction, myoglobin increases within 1–3 hours of the infarct. Myoglobin is not cardiac specific, and increased serum levels also occur in vigorous exercise, intramuscular injections, rhabdomyolysis, and muscular dystrophy. Because myoglobin is a relatively small protein and able to be excreted by the kidneys, elevated serum levels occur in renal failure.

239.

D. Troponin is a group of three proteins that function in muscle contraction by binding to the thin filaments of cardiac and skeletal striated muscle. The three proteins are known as troponin T (TnT), troponin I (TnI), and troponin C (TnC). With AMI, the cardiac-specific isoforms of troponin are released into the blood; the two of clinical interest are cTnI and cTnT. Cardiac troponin I (cTnI) will show an increase that exceeds the reference interval in approximately 3–6 hours following an AMI. Quantification should be done serially starting with an initial measurement at presentation followed by testing at 3–6 hours, 6–9 hours, and 12–24 hours. cTnI will remain elevated for 5–10 days. Unlike cTnT, which is expressed in small quantities in regenerating and diseased skeletal muscle, cTnI is not, which makes it specific for cardiac muscle.

240.

C. For many years, the diagnosis of an AMI was facilitated by assaying serum levels of aspartate aminotransferase (AST), lactate dehydrogenase (LD), creatine kinase (CK), and LD and CK isoenzymes. Today the clinical usefulness of AST and LD has been replaced primarily by cardiac troponin and to a lesser degree by myoglobin, whereas CK isoenzymes continue to play a role. Although myoglobin will increase above the upper reference interval in 1–3 hours following AMI, it is not tissue specific for cardiac muscle and its application has found limited usefulness. Myoglobin will also be increased following skeletal muscle trauma. Troponin I and troponin T have proven to be useful markers, because each has a cardiac-specific isoform, cTnI and cTnT. cTnI appears to be more specific for cardiac muscle, because it has not been identified in regenerating or diseased skeletal muscle, whereas cTnT is made in small amounts by skeletal muscle. Total CK is elevated in AMI and takes 4–6 hours to rise above the upper reference interval. It is the increased level of CK-2 (CK-MB) that is more helpful in diagnosing AMI, but caution needs to be exercised here, because skeletal muscle injury can cause a similar increase.

241.

D. Osteitis deformans, also known as Paget disease, is a chronic disorder of bone. This disorder is characterized by a significant increase in the serum alkaline phosphatase level. Gamma-glutamyltransferase will be normal in bone disease, because this enzyme is not found in bone tissue. However, in hepatobiliary disease both enzymes would characteristically be elevated.

242.

C. Obstruction of the biliary tree is also referred to as intrahepatic cholestasis. This disorder is characterized by significant elevations in the serum levels of alkaline phosphatase and gamma-glutamyltransferase. The serum levels of alanine and aspartate aminotransferases and lactate dehydrogenase are only slightly elevated. Early in the disease, the serum bilirubin level may be normal or only slightly elevated. In alcoholic cirrhosis, viral hepatitis, and infectious mononucleosis, only a slight to moderate elevation of alkaline phosphatase would be seen.

243.

A. Acute hepatitis is characterized by markedly elevated levels of serum alanine aminotransferase and aspartate aminotransferase, which may range from 10- to 100-fold greater than the reference values. Although alkaline phosphatase and gamma-glutamyltransferase are increased, their elevations are less notable than the aminotransferases. Alkaline phosphatase may range up to two times the reference range whereas gamma-glutamyltransferase may go as high as five times the reference range in acute hepatitis. Due to leakage of conjugated bilirubin from the hepatocytes, the urine bilirubin will be positive. With less conjugated bilirubin reaching the intestines, fecal urobilinogen will be less than normal.

244.

A. To aid in the diagnosis of skeletal muscle disease, measurement of creatine kinase would be most useful. CK yields the most reliable information when skeletal muscle disease is suspected. Other enzymes that are also useful to measure are aspartate aminotransferase and lactate dehydrogenase. Both of these enzymes will be moderately elevated, whereas CK is significantly increased.

245.

C. When an AMI occurs, CK is the first enzyme to become elevated in the blood, rising within 4 to 6 hours following chest pain. AST exhibits a rise in the serum level within 6 to 8 hours. LD shows an increase in 8 to 12 hours following infarction. Measurement of these three enzymes to assess acute myocardial infarction has been replaced by cardiac troponin, myoglobin, and CK-MB. However, awareness of the CK, AST, and LD patterns as well as other biochemical tests is useful in assessing organ complications that may arise during the period of AMI.

246.

C. Quantification of serum total creatine kinase, CK-MB (or CK-2) isoenzyme, and cardiac troponin I (cTnI) or cardiac troponin T (cTnT) is very useful in determining an AMI. Determining the presence and activity level of CK-MB is valuable, because CK-MB levels can increase following an infarct, ranging from 6 to 30% of the total CK. Serial assessment of serum specimens is recommended, with the initial specimen obtained at presentation, followed by blood collection at 3–6 hours, 6–9 hours, and 12–24 hours from the initial time. Because alkaline phosphatase isoenzymes are associated with liver, bone, intestinal, and placental tissues, their analysis would not contribute any significant information to determining the occurrence of an AMI.

247.

D. Symptoms are sometimes nonspecific, making it difficult to diagnose congestive heart failure. B-type (brain) natriuretic peptide (BNP) is used to determine if physical symptoms are related to congestive heart failure. BNP is synthesized in and secreted by myocardial ventricles in response to ventricular volume expansion and pressure overload. An increase in BNP causes dilation of blood vessels and promotes sodium and water loss by the kidneys. This reduces fluid load on the heart in an attempt to improve cardiac function. Albumin cobalt binding is a test that measures ischemia-modified albumin, which is a marker for ischemic heart disease.

248.

B. The child's symptoms are consistent with Duchenne dystrophy, which is an X-linked recessive disorder. It is characterized by muscle weakness, which is caused by destruction of muscle fibers. Symptoms are seen in male children starting at 3 to 7 years of age. The most notable enzyme increase is in creatine kinase, which may increase 50–100 times the reference range. Aspartate transaminase and lactate dehydrogenase would also be increased, because both enzymes are present in skeletal muscle tissue. Alkaline phosphatase is not present in skeletal muscle tissue and is measured to assess hepatobiliary and bone disorders.

249.

B. The controls were within acceptable limits, so it is assumed that all test results are accurate. The elevated myoglobin, total CK, and CK-MB with a troponin I that showed no change and remained in the reference range suggest that the elevated results were due to the skeletal muscle injuries sustained in the car crash. Myoglobin is not tissue specific and may be increased in skeletal muscle injuries, muscular dystrophy, and AMI. The same is true for creatine kinase, which is not tissue specific and may be increased in skeletal muscle disorders as well as cardiac muscle disorders. CK-MB, although it is associated with cardiac muscle and the occurrence of AMI, is not tissue specific and will increase with skeletal muscle injury (but to a lesser degree than CK-MM). Cardiac troponin I is tissue specific and not expressed by skeletal muscle; thus it would remain within the reference range in the absence of an AMI. Total CK and CK-MB do not provide information to assess if a stroke has occurred.

250.

A. Elevated homocysteine levels are associated with increased risk for coronary heart disease. Increased homocysteine contributes to the damage of arterial walls preceding formation of plaques. Individuals at risk need to be evaluated for vitamin B levels, because low levels of folic acid, vitamin B_6, and vitamin B_{12} are associated with increased levels of homocysteine.

251.

C. N-terminal proBNP is released when BNP (B-type or brain natriuretic peptide) is cleaved from precursor proBNP. NT-proBNP is released in a 1:1 ratio to BNP; however, NT-proBNP has a half-life of hours as compared to BNP's short half-life of approximately 22 minutes. The longer half-life of NT-proBNP contributes to its clinical utility. In addition, when measuring NT-proBNP, there is no interference when an individual is being treated with nesiritide, which is human recombinant BNP used in treating congestive heart failure.

252.

D. Reye syndrome is associated with viral infections, exogenous toxins, and salicylate use. The disorder generally manifests itself in children from 2 to 13 years of age. The laboratory findings that support a diagnosis of Reye syndrome include increased levels of serum aspartate and alanine transaminases (greater than 3 times the reference range), increased plasma ammonia level (can exceed 100 μg/dL), and prolonged prothrombin time (3 sec or more than the control). In Reye syndrome the serum bilirubin level is generally within the reference range.

253.

C. Lipase activity can be determined using a dilute olive oil emulsion as the substrate. The fatty micellar complexes absorb light as well as scatter light. Lipase catalyzes the hydrolysis of these triglyceride complexes, forming fatty acid and glycerol products. With the degradation of the micellar complexes, clearing of the reagent mixture occurs, causing changes in turbidity and light scatter. The rate at which the turbidity decreases can be monitored spectrophotometrically at 400 nm, or the decrease in light scatter can be measured using a nephelometer. The rate of these changes can be equated to the lipase activity present in the serum specimen.

254.

D. Cystic fibrosis is inherited as an autosomal recessive trait. It is a systemic disease that affects the exocrine glands, causing gastrointestinal malabsorption, pancreatic insufficiency, and pulmonary disease. Cystic fibrosis is characterized by increased concentrations of chloride and sodium in sweat. With pancreatic insufficiency, the amount of lipase, amylase, trypsin, and bicarbonate secreted into the duodenum is decreased. Because the three enzymes contribute to digestion of fats, starches, and proteins, respectively, children with this disorder suffer from malabsorption.

Liver Function and Porphyrin Formation

255.

D. The biochemical synthesis of the porphyrins consists of a series of reactions. Succinyl coenzyme A and glycine are the two compounds that originally condense to form aminolevulinic acid (ALA). Through a second condensation reaction, two molecules of ALA condense and cyclize to form porphobilinogen. Porphobilinogen is a monopyrrole structure and the precursor of porphyrin synthesis.

256.

D. Heme is derived from a series of biochemical reactions that begin with the formation of porphobilinogen from succinyl coenzyme A and glycine. Because porphobilinogen is a monopyrrole, four molecules of porphobilinogen condense and cyclize to form the porphyrinogen precursors of protoporphyrin IX. Protoporphyrin IX chelates iron to form heme and is, therefore, the immediate precursor of heme formation.

257.

D. Porphobilinogen is a precursor compound in the biosynthesis of heme. In acute intermittent porphyria, excess amounts of porphobilinogen are excreted in the urine. The Watson-Schwartz test employs *p*-dimethylaminobenzaldehyde reagent (also known as Ehrlich's aldehyde reagent) to form a red condensation product with porphobilinogen.

258.

D. The porphyrins that are of clinical significance include uroporphyrin, coproporphyrin, and protoporphyrin. These three porphyrin compounds may be detected in acid solution by irradiating the solution with long-wave ultraviolet light, which causes the porphyrins to fluoresce. The intense orange-red fluorescence of the porphyrins is due to the conjugated unsaturation of the tetrapyrrole ring structure.

259.

B. When measurement of aminolevulinic acid, porphobilinogen, uroporphyrin, or coproporphyrin is requested, a 24-hour urine specimen should be collected. The urine should be refrigerated during collection and stored in a brown bottle to protect light-sensitive compounds. Because porphobilinogen is more stable under alkaline conditions and aminolevulinic acid is more stable under acid conditions, sodium bicarbonate should be added as a compromise to maintain the pH near 7.

260.

D. In the catabolic process of hemoglobin degradation, the alpha-carbon methene bridge of the tetrapyrrole ring structure of heme opens oxidatively to form verdohemoglobin. Verdohemoglobin is a complex composed of biliverdin, iron, and the protein globin. This complex then undergoes degradation in which iron is removed and returned to the body iron stores, the globin portion is returned to the amino acid pool, and the biliverdin undergoes reduction to form bilirubin. It is biliverdin, therefore, that is the immediate precursor of bilirubin formation. Mesobilirubinogen and urobilinogen represent intestinal breakdown products of bilirubin catabolism.

261.

B. Diazo reagent is a mixture of sulfanilic acid, sodium nitrite, and hydrochloric acid. The mixing of sodium nitrite with hydrochloric acid forms nitrous acid, which in turn reacts with sulfanilic acid to form a diazonium salt. This diazotized sulfanilic acid mixture, when mixed with solubilized bilirubin, forms a red azobilirubin complex. The azobilirubin complexes are isomeric structures formed from the splitting of the bilirubin compound in half. Each half then reacts with the diazo reagent to form two isomeric azobilirubin complexes.

262.

C. In order for the bilirubin-albumin complex to reach the parenchymal cells of the liver, the complex must be transported from the sinusoids to the sinusoidal microvilli and into the parenchymal cell. The microsomal fraction of the parenchymal cell is responsible for the conjugation of bilirubin. It is here that bilirubin reacts with uridine diphosphate glucuronate in the presence of the enzyme uridine diphosphate glucuronyltransferase to form bilirubin diglucuronide.

263.

B. Bilirubin that has been secreted through the bile into the small intestine is reduced by anaerobic microorganisms to urobilinogen. One of the possible fates of urobilinogen is its conversion to urobilin. In the colon, a portion of the urobilinogen is oxidized by the action of microorganisms to urobilin, which is excreted in the feces as an orange-brown pigment.

264.

A. The cells of the reticuloendothelial system are able to phagocytize aged red blood cells and convert the hemoglobin to the excretory product bilirubin. It is then necessary for the bilirubin to be transported to the liver, where it is conjugated for excretion in the bile. Albumin acts as the transport vehicle for unconjugated bilirubin in the blood, with each mole of albumin capable of binding two moles of bilirubin.

265.

A. When total bilirubin levels exceed 2.5 mg/dL, the clinical manifestation of jaundice develops. Characteristically, such body areas as the skin and sclera develop a yellow-pigmented appearance. Jaundice may be caused by an increase in either the unconjugated or conjugated form of bilirubin. Such increases in bilirubin levels may be caused by prehepatic, hepatic, or posthepatic disorders.

266.

A. An abnormal accumulation of bilirubin in the body may be due to increased production or decreased excretion of bilirubin. Terms frequently associated with a buildup of bilirubin include "jaundice," "kernicterus," and "icterus." Both jaundice and icterus are characterized by the yellow coloration of the skin, sclera, and mucous membranes that results from increased plasma concentrations of either conjugated or unconjugated bilirubin or both. This yellow coloration is also visible in serum and plasma specimens *in vitro*. *Kernicterus* refers to the accumulation of bilirubin in brain tissue that occurs with elevated levels of unconjugated bilirubin. This condition is most commonly seen in newborns with hemolytic disease resulting from maternal-fetal Rh incompatibility. Newborns afflicted with kernicterus will exhibit severe neural symptoms.

267.

B. In the small intestine, urobilinogen is formed through the enzymatic reduction process of anaerobic bacteria on bilirubin. The fate of urobilinogen is such that some of the urobilinogen will be excreted unchanged in the stool, a portion will be oxidized to urobilin for excretion in the stool, and up to 20% will be absorbed from the intestine into the portal circulation. This circulating urobilinogen is almost completely picked up by the liver, with only a small amount excreted in the urine. The liver oxidizes a small part of the recycled urobilinogen to bilirubin. This newly formed bilirubin and any unchanged urobilinogen are transported through the bile canaliculi into the bile for reexcretion by the intestines. This recycling of urobilinogen is part of the enterohepatic circulation.

268.

D. Bilirubin will deteriorate when exposed to either white or UV light. This deterioration is also temperature sensitive. Thus, specimens for bilirubin analysis should be stored in the dark at refrigerator temperature until the assay can be performed. Lipemia should be avoided, due to its interference with spectrophotometric analyses. Because hemoglobin reacts with diazo reagent, use of hemolyzed specimens should be avoided. Hemolysis will cause bilirubin results to be falsely low.

269.

C. Four bilirubin fractions represented by Greek letters have been identified: unconjugated (alpha), monoconjugated (beta), diconjugated (gamma), and unconjugated bilirubin covalently bound to albumin (delta). Delta-bilirubin is normally present in low concentration in the blood, and it is known to react directly with diazotized sulfanilic acid. Increased serum levels of delta-bilirubin are associated with liver-biliary disease.

270.

A. The cells of the reticuloendothelial system are responsible for the removal of old red blood cells from the peripheral circulation. As the red blood cells reach the end of their 120-day life span, the specialized cells mainly of the spleen phagocytize the aged cells and convert the released hemoglobin into the excretory pigment bilirubin. The bone marrow is also responsible for the destruction of a small number of red blood cells that have not completed the maturation process. The bilirubin produced by the reticuloendothelial cells is indirect bilirubin, which, as a protein-bound compound, is transported to the liver for conjugation into direct bilirubin.

271.

C. Bilirubinometer, bilirubin oxidase, and Jendrassik-Grof are methods that have been used to quantify serum bilirubin concentrations. The bilirubinometer is used for direct spectrophotometric assay in which the bilirubin concentration is read directly at 454 nm. In the bilirubin oxidase method, bilirubin is oxidized to biliverdin and the reaction is followed at 405–460 nm. The Jendrassik-Grof method utilizes a caffeine-sodium benzoate mixture to accelerate the coupling reaction of unconjugated bilirubin with diazo reagent to form an azobilirubin complex. Because of a high recovery rate, the Jendrassik-Grof method is considered to be the method of choice for bilirubin analysis.

272.

A. Direct bilirubin was so named because of its ability in the van den Bergh method to react directly with diazotized sulfanilic acid without the addition of alcohol. Such a direct reaction is possible because direct bilirubin is conjugated in the liver with glucuronic acid, thereby making it a polar, water-soluble compound. Because conjugated bilirubin is both water soluble and not protein bound, it may be filtered through the glomerulus and excreted in the urine of jaundiced patients. Indirect bilirubin is a protein-bound unconjugated compound that is soluble in alcohol but not in water, and because of these properties, it is unable to be excreted in the urine.

273.

C. Ehrlich's diazo reagent consists of sulfanilic acid, hydrochloric acid, and sodium nitrite. Sulfanilic acid is dissolved in hydrochloric acid and diluted to volume with deionized water. Sodium nitrite is dissolved in deionized water and diluted to volume. Aliquots of these two reagent mixtures are combined to prepare Ehrlich's diazo reagent, which must be prepared fresh before use because of its unstable nature.

274.

C. Unlike direct bilirubin, indirect-reacting bilirubin is insoluble in deionized water and dilute hydrochloric acid. Indirect-reacting bilirubin must first be mixed with methanol or caffeine-sodium benzoate to solubilize it before proceeding with the diazo reaction. Because of these properties, total bilirubin and direct bilirubin are usually chemically analyzed, and the indirect, or unconjugated, fraction is calculated from the difference between the total and direct values. The total value represents the reaction of both conjugated and unconjugated bilirubin, whereas the direct value represents only the reaction of conjugated bilirubin.

275.

B. In the bilirubin oxidase method, the enzyme bilirubin oxidase catalyzes the oxidation of bilirubin to the product biliverdin, which is colorless. This is seen as a decrease in absorbance and is monitored between 405 and 460 nm. This method has an advantage over diazo methods in that hemoglobin does not interfere in the assay and cause falsely low results.

276.

A. Conjugated bilirubin and a small amount of unconjugated bilirubin will pass from the bile into the small intestine. In the small intestine, enzyme systems of anaerobic bacteria are able to reduce bilirubin to the reduction products mesobilirubinogen, stercobilinogen, and urobilinogen. These three reduction products of bilirubin catabolism are collectively referred to as urobilinogen.

277.

D. "Obstructive jaundice" is a term applied to conditions in which the common bile duct is obstructed because of gallstone formation, spasm, or neoplasm. Such an obstruction blocks the flow of bile from the gallbladder into the small intestine. This impedance of bile flow will result in a backflow of bile from the gallbladder into the sinusoids of the liver and ultimately into the peripheral circulation. Because the liver is not initially involved and the disorder is of posthepatic origin, the increased levels of bilirubin in the blood are caused by the backflow of conjugated bilirubin. If the disorder is allowed to progress, the continued backflow of bile will cause parenchymal cell destruction. Such cellular necrosis will result in a depression of the conjugating ability of the liver, and an elevation of unconjugated bilirubin levels in the blood will ensue.

278.

A. Hemolytic jaundice is also referred to as prehepatic jaundice. It is caused by excessive destruction of erythrocytes at a rate that exceeds the conjugating ability of the liver. As a result, increased levels of unconjugated bilirubin appear in the blood. The amount of conjugated bilirubin being formed in the liver is proportionately greater than normal; this is reflected in the increased levels of urobilinogen and urobilin found in the stool. Because of the enterohepatic circulation, the increased urobilinogen levels in the small intestines are reflected by an increase in the circulating blood levels of urobilinogen. Because the liver is unable to pick up all the circulating urobilinogen, the urinary levels of urobilinogen are increased. Urinary bilirubin levels are negative because the blood level of conjugated bilirubin is usually normal.

279.

D. The enzyme uridine diphosphate glucuronyltransferase catalyzes the conjugation of bilirubin with glucuronic acid. In newborns, especially premature infants, this liver enzyme system is not fully developed or functional. Because of this deficiency in the enzyme system, the concentration of unconjugated bilirubin rises in the blood, because only the conjugated form may be excreted through the bile and urine. The increased levels of unconjugated bilirubin will cause the infant to appear jaundiced. Generally, this condition persists for only a short period because the enzyme system usually becomes functional within several days after birth. Neonatal physiological jaundice resulting from an enzyme deficiency is hepatic in origin. Hemolytic jaundice resulting from either Rh or ABO incompatibility is a prehepatic type of jaundice, whereas a stricture of the common bile duct is classified as posthepatic jaundice.

280.

C. With complete obstruction of the common bile duct, bilirubin diglucuronide would be unable to pass from the bile into the intestines. Such obstruction to the flow of bile will cause the conjugated bilirubin to be regurgitated into the sinusoids and the general circulation. Because conjugated bilirubin is water soluble, it will be excreted in the urine. However, because of the lack of bile flow into the intestines, neither urobilinogen nor urobilin will be present in the feces. The lack of urobilin in the feces will be apparent from the light brown to chalky-white coloration of the stools. Because there is no urobilinogen in the intestines to be picked up by the enterohepatic circulation, the urinary excretion of urobilinogen will be negative. Because the obstruction may sometimes be only partial, this description would be somewhat altered. Provided that some bile was able to flow into the intestines, the fecal urobilinogen and urobilin concentrations would be present but depressed, the urinary urobilinogen excretion would be below normal, and the urinary bilirubin level would be increased.

281.

B. In disorders such as viral hepatitis, toxic hepatitis, and cirrhosis, hepatocellular damage occurs. The damaged parenchymal cells lose their ability either to conjugate bilirubin or to transport the bilirubin that is conjugated into the bile. Because of loss of conjugating ability by some parenchymal cells, the early stage of viral hepatitis is characterized by an increase in the unconjugated bilirubin fraction in the blood. An increase of lesser magnitude in the conjugated fraction is also demonstrated. The increase in conjugated bilirubin is due to the fact that some cells are able to conjugate but are damaged in such a way that there is leakage of conjugated bilirubin into the sinusoids and the general circulation. Because of this increase in the conjugated fraction, urinary bilirubin excretion is positive. Because the amount of conjugated bilirubin reaching the intestines is less than normal, it follows that the fecal urobilinogen and urobilin levels will also be less than normal. However, the urinary urobilinogen levels will be greater than normal because the urobilinogen that does reach the enterohepatic circulation is not efficiently removed by the liver but, rather, is excreted by the urinary system.

282.

B. Both Crigler-Najjar syndrome and neonatal jaundice, a physiological disorder, are due to a deficiency in the enzyme-conjugating system. With a deficiency in uridine diphosphate glucuronyltransferase, the liver is unable to conjugate bilirubin, and both of these conditions are characterized by increased levels of unconjugated bilirubin. Unlike Crigler-Najjar syndrome, which is a hereditary disorder, neonatal physiological jaundice is a temporary situation that usually corrects itself within a few days after birth.

283.

D. Gilbert syndrome is a preconjugation transport disturbance. In this disorder the hepatic uptake of bilirubin is defective because the transportation of bilirubin from the sinusoidal membrane to the microsomal region is impaired. Gilbert syndrome is inherited as an autosomal dominant trait characterized by increased levels of unconjugated bilirubin.

284.

C. In Dubin-Johnson syndrome, the transport of conjugated (direct) bilirubin from the microsomal region to the bile canaliculi is impaired. In this rare familial disorder, plasma conjugated bilirubin levels are increased because of defective excretion of bilirubin in the bile. Because conjugated bilirubin is water soluble, increased amounts of bilirubin are found in the urine.

285.

D. Abnormal conditions characterized by jaundice may be classified according to their type of liver involvement. The three types of jaundice are prehepatic, hepatic, and posthepatic. Hepatic jaundice may be subdivided into two groups on the basis of the type of excessive bilirubin: conjugated bilirubin or unconjugated bilirubin. Gilbert syndrome and Dubin-Johnson syndrome are disorders in which the process of bilirubin transport is malfunctioning. Both Crigler-Najjar syndrome and neonatal jaundice, a physiological disorder, are due to a deficiency in the enzyme-conjugating system. Disorders such as viral hepatitis, toxic hepatitis, and cirrhosis cause damage and destruction of liver cells so that the ability of the liver to remove unconjugated bilirubin from the blood and to conjugate it with glucuronic acid becomes impaired. As these disorders progress, the level of unconjugated bilirubin in the blood rises. There is also an increase, although not as great as that of unconjugated bilirubin, in blood levels of conjugated bilirubin. The cause is a leakage of conjugated bilirubin from damaged parenchymal cells into the sinusoids. Neoplasm of the common bile duct is a form of posthepatic jaundice.

286.

A. Prehepatic jaundice is also known as hemolytic jaundice, a term that is descriptive of the cause of the disorder. Any disorder that causes the destruction of erythrocytes at a faster rate than the liver is able to conjugate the bilirubin being formed by the reticuloendothelial system will exhibit hyperbilirubinemia. The increased concentration of bilirubin and the ensuing jaundice is not due to any hepatic malfunction but only to the inability of the liver to handle the conjugation of such a bilirubin overload. Therefore, the jaundice is caused by an increased concentration of unconjugated bilirubin. Disorders that follow this type of course are acute hemolytic anemia, chronic hemolytic anemia, and neonatal jaundice. Causes of hemolytic anemia may be genetic or acquired and include hereditary spherocytosis, sickle-cell anemia, and blood transfusion reactions. Neonatal jaundice may be due to an ABO or Rh incompatibility, as seen in erythroblastosis fetalis.

287.

B. Posthepatic jaundice is caused by an obstruction in the common bile duct, extrahepatic ducts, or the ampulla of Vater. Such an obstruction may be caused by gallstones, neoplasms, or strictures. In this type of jaundice, the liver is functioning properly in its conjugation of bilirubin, but the obstruction causes a blockage so that the conjugated bilirubin is unable to be excreted through the intestines. Therefore, there is a backup of bile into the sinusoids and an overflow into the blood. The circulating blood will characteristically contain excessive amounts of conjugated bilirubin, which will cause increased amounts of bilirubin to be excreted in the urine. Because the blockage prevents proper excretion of bilirubin into the intestines, the formation of urobilinogen and urobilin is impeded. This pattern will continue until the regurgitation of bile causes hepatocellular damage. With destruction of the parenchymal cells, conjugation of bilirubin will be depressed and the blood levels of unconjugated bilirubin will also rise.

288.

D. The laboratory test results suggest that the woman has posthepatic biliary obstruction. The diagnosis is supported by the greater increase in alkaline phosphatase in contrast to the lesser increases in ALT and AST, the greater increase in the direct (conjugated) bilirubin level, and the decrease in urine urobilinogen. In biliary obstruction, increased synthesis of alkaline phosphatase is induced, with more produced in hepatocytes adjacent to biliary canaliculi. When an obstruction occurs in the biliary system, which may be caused by such disorders as gallstones in the common bile duct or a tumor in the region of the ampulla of Vater, the conjugated bilirubin made in the liver is unable to pass into the intestines. This will increase the bilirubin level in the blood, as well as result in less production of urobilinogen in the intestines. Thus, less urobilinogen will be transported in the enterohepatic circulation and less urobilinogen will be excreted in the urine. The other choices, viral hepatitis, exposure to toxic chemicals, and cirrhosis, are hepatic disorders that affect the hepatocytes directly and thus liver function. In such cases, there would be hepatocyte injury or tissue necrosis resulting in greater elevation of ALT and AST as compared to alkaline phosphatase, and the unconjugated bilirubin would be the fraction with the more significant increase because hepatocyte function is compromised. Urobilinogen would also be increased in the urine because of the inability of the liver to process it.

289.

A. In viral hepatitis, hepatocyte injury and necrosis cause the release of cellular contents. ALT is more specific for hepatocyte injury because it is significantly present in liver tissue, whereas AST is less specific because of its significant presence not only in liver but also in many other tissues. In viral hepatitis, both ALT and AST are significantly elevated in serum. Because liver function is compromised in viral hepatitis, the liver will be unable to pick up urobilinogen from the enterohepatic circulation to process, resulting in increased urobilinogen excretion in the urine. Although serum total bilirubin will be elevated, the indirect bilirubin (unconjugated) will comprise the larger fraction and the direct bilirubin (conjugated) will be the lesser fraction. Liver function is compromised, as is the ability of the liver to pick up bilirubin and conjugate it.

Electrolytes and Osmolality

290.

C. Sodium is the principal cation found in the plasma. The normal serum sodium level is 136–145 mmol/L, whereas in urine the sodium concentration ranges between 40 and 220 mmol/day, being dependent on dietary intake. Because sodium is a threshold substance, it is normally excreted in the urine when the serum sodium concentration exceeds 110–130 mmol/L. When serum levels fall below 110 mmol/L, all the sodium in the glomerular filtrate is virtually reabsorbed in the proximal and distal tubules. This reabsorption process is influenced by the hormone aldosterone.

291.

D. Osmolality is a measure of the total number of solute particles per unit weight of solution and is expressed as milliosmoles per kilogram of water. The normal osmolality of serum is in the range of 275–295 mOsm/kg water. For monovalent cations or anions the contribution to osmolality is approximately 92%. Other serum electrolytes, serum proteins, glucose, and urea contribute to the remaining 8%.

292.

B. Hemolysis of blood specimens because of physiological factors is often difficult to differentiate from hemolysis produced by the blood collection itself. In either case, the concentration of potassium will be increased in the serum because of the release of the very high level of intracellular potassium from the erythrocytes into the plasma. When hemolysis is present, the serum concentrations of sodium, bicarbonate, chloride, and calcium will be decreased because their concentrations are lower in erythrocytes than in plasma.

293.

D. The largest fractions of the anion content of serum are normally provided by chloride and bicarbonate. The third largest anion fraction is contributed by the proteins that are negatively charged at physiological pH and that provide about 16 mmol ion charge per liter. Of the remaining organic anions, the largest contribution is generally from lactate, which ranges normally from 1 mmol/L up to 25 mmol/L in lactic acidosis. The ketone bodies, including acetoacetate, normally constitute only a small fraction of the total anions, but their total contribution may increase to 20 mmol/L in diabetic acidosis. Iron is present in the serum as a cation and does not contribute.

294.

D. In contrast to sodium, which is the principal plasma cation, potassium is the principal cellular cation. After absorption in the intestinal tract, potassium is partially filtered from the plasma by the kidneys. It is then almost completely reabsorbed from the glomerular filtrate by the proximal tubules and subsequently reexcreted by the distal tubules. Unlike sodium, potassium exhibits no renal threshold, being excreted into the urine even in K^+-depleted states. In acidotic states, as in renal tubular acidosis in which the exchange of Na^+ for H^+ is impaired, the resulting retention of potassium causes an elevation in serum K^+ levels. Hemolysis must be avoided in blood specimens that are to be used for K^+ analysis because erythrocytes contain a potassium concentration 23 times greater than serum K^+ levels. If the red blood cells are hemolyzed, a significant increase in serum K^+ will result.

295.

A. Chloride can be quantified by the spectrophotometric ferric perchlorate method. The reagent reacts with chloride to form a colored complex. Other methods employed are the spectrophotometric mercuric thiocyanate method, the coulometric-amperometric titration method, and the ion-selective electrode method, which is employed by many automated analyzers.

296.

C. Chloride is the principal plasma anion. The average concentration of chloride in plasma is 103 mmol/L. In the kidneys, chloride ions are removed from the blood through the glomerulus and then passively reabsorbed by the proximal tubules. The chloride pump actively reabsorbs chloride in the thick ascending limb of the loop of Henle. In the lungs, chloride ions participate in buffering the blood by shifting from the plasma to the red blood cells to compensate for ionic changes that occur in the alveoli when the HCO_3^- from the red blood cells enters the plasma. This is termed the chloride shift. Chloride can be measured in a variety of body fluids, including serum, plasma, urine, and sweat.

297.

A. The calculation of the anion gap may be used both to assess instrument performance and as a quality assurance tool for electrolyte analyses. The following is one of several equations that may be used to calculate the anion gap: anion gap (mmol/L) = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$. The acceptable reference range for this method of calculation is 10–20 mmol/L. If the values of a particular patient fall within this acceptable level, it is presumed that there are no gross problems with the electrolyte measurements. In this case, the anion gap is 18 mmol/L and within the reference range. When using the anion gap it is important to remember that values are affected not only by measurement errors but also by such disease processes as renal failure, ketoacidosis, and salicylate poisoning. Therefore, it is important to differentiate between laboratory errors and true disease states.

298.

C. Addison disease is characterized by the hyposecretion of the adrenocortical hormones by the adrenal cortex. Both aldosterone, a mineralocorticoid, and cortisol, a glucocorticoid, are inadequately secreted in this disorder. The decreased secretion of aldosterone will affect body electrolyte balance and extracellular fluid volume. The decrease in sodium reabsorption by the renal tubules will be accompanied by decreased chloride and water retention. This loss of sodium, chloride, and water into the urine will cause the extracellular fluid volume to be decreased. Additionally, the decreased reabsorption of sodium will interfere with the secretion of potassium and hydrogen ions in the renal tubules, causing an increase in the serum potassium ion and hydrogen ion (acidosis) concentrations.

299.

D. Primary aldosteronism is characterized by the hypersecretion of aldosterone, a mineralocorticoid, by the zona glomerulosa cells of the adrenal cortex. Excessive secretion of aldosterone will increase renal tubular reabsorption of sodium, resulting in a decrease in the loss of sodium in the urine. The net result of this mechanism is increased sodium in the extracellular fluid. Additionally, there will be increased renal excretion of potassium, causing a decrease of potassium in the extracellular fluid.

300.

D. A decreased serum sodium concentration, or hyponatremia, is associated with a variety of disorders, including (1) *Addison disease,* which involves the inadequate secretion of aldosterone, resulting in decreased reabsorption of sodium by the renal tubules; (2) *diarrhea,* which involves the impaired absorption from the gastrointestinal tract of dietary sodium and of sodium from the pancreatic juice, causing an excessive quantity of sodium to be excreted in the feces; (3) *diuretic therapy,* which causes a loss of water with concurrent loss of electrolytes, including sodium; and (4) *renal tubular disease,* which involves either the insufficient reabsorption of sodium in the tubules or a defect in the Na^+–H^+ tubular exchange mechanism. A diagnosis of Cushing syndrome is incorrect because the disorder is associated with hypernatremia.

301.

C. Free ionized calcium normally accounts for about 50% of total serum calcium, with the remainder being made up of complexed calcium (about 10%) and calcium bound to proteins (about 40%). The main factors that affect the free ionized calcium fraction are the protein concentration and the pH of the blood. Calcium ions are bound mainly to albumin, but they also bind to globulins. Because the binding is reversible, factors that decrease the protein concentration will increase the free ionized fraction of calcium in the blood. A decrease in blood pH will also increase the fraction of free ionized calcium.

302.

D. The renal tubular reabsorption of phosphate is controlled by the action of parathyroid hormone (PTH) on the kidney. Increased PTH secretion from any cause will lead to a decreased tubular reabsorption of phosphate (increased urine phosphate and decreased serum phosphate). The test is useful in distinguishing serum hypercalcemia that is a result of excess PTH production by the parathyroid glands from hypercalcemia due to other causes (e.g., bone disease).

303.

D. PTH, calcitonin, vitamin D, plasma proteins, and plasma phosphates are factors that influence plasma calcium levels. *PTH* is a hormone important in maintaining plasma calcium levels. It mobilizes calcium from bones. It increases the synthesis of one of the vitamin D derivatives, thereby causing an increase in bone resorption and intestinal absorption of calcium. When normal calcium levels are restored, PTH secretion is cut off (negative-feedback mechanism). *Calcitonin* (thyrocalcitonin) is a hormone secreted by the thyroid gland in response to elevated levels of plasma calcium. It acts by inhibiting bone resorption of calcium, thereby preventing significant variations in plasma calcium concentrations. Hydroxylation of *vitamin D* gives a derivative that will increase the intestinal absorption of calcium and phosphates.

304.

D. PTH has physiological actions on bone, kidney, and intestine. Its overall effect is to raise serum ionized calcium levels and lower serum phosphorus levels. Its actions on various organs are the result of a combination of both direct and indirect effects. In bones, PTH directly acts to increase bone resorption, thereby increasing both calcium and phosphorus in the blood. In the kidneys, PTH directly acts on the renal tubules to decrease phosphate reabsorption. In combination with the effect on bone, the overall result is a decrease in blood phosphorus levels. In the intestines, PTH acts to increase absorption of calcium by its action in increasing 1,25-dihydroxyvitamin D_3 synthesis in the kidneys, which in turn stimulates intestinal absorption of calcium.

305.

A. Primary hyperparathyroidism is a disorder characterized by increased secretion of PTH into the blood, without the stimulus on the parathyroid gland of a decreased level of ionized calcium. The increase in PTH produces increased blood calcium and vitamin D_3 levels, along with a decreased blood phosphorus level. The hypersecretion is most often caused by a single parathyroid adenoma. PTH secretion can usually, but not in all cases, be suppressed by calcium infusion. The decreased blood phosphate level is a result of the action of PTH on the kidneys, which decreases tubular reabsorption of phosphate ions. The increased blood level of 1,25-dihydroxyvitamin D_3 is also caused by PTH action on the kidneys in that PTH stimulates increased renal synthesis of this compound.

306.

C. Secondary hyperparathyroidism is a disorder that represents the response of a normally functioning parathyroid gland to chronic hypocalcemia. In most patients, the hypocalcemia is the result of renal disease or vitamin D deficiency. Vitamin D deficiency decreases intestinal calcium absorption, resulting in hypocalcemia. The hypocalcemia resulting from renal disease is more complex. It can result either from the increased serum phosphate level caused by decreased glomerular filtration or from the decreased synthesis of 1,25-dihydroxyvitamin D_3 in kidney disease.

307.

B. Serum inorganic phosphate concentrations are determined most commonly by reacting with ammonium molybdate reagent. The molybdenum-phosphate complexes can be quantified at 340 nm. Alternately, treatment of the phosphomolybdate compound formed with a reducing agent leads to the formation of molybdenum blue, which can be measured spectrophotometrically. Use of the anticoagulants EDTA, oxalate, and citrate should be avoided, because they interfere with the formation of phosphomolybdate.

308.

A. Total serum calcium concentration is often determined by the spectrophotometric quantification of the color complex formed with cresolphthalein complexone. Magnesium will also form a color complex and, therefore, is removed by reacting the serum with 8-hydroxyquinoline. Calcium concentration is determined with the use of a variety of other reagents and most reliably by means of atomic absorption spectrophotometry.

309.

B. Plasma phosphates influence plasma calcium levels. Case studies show that there is a reciprocal relationship between calcium and phosphorus. A decrease in plasma calcium will be accompanied by an increase in plasma inorganic phosphate.

310.

B. Similarly to potassium, which is a major intracellular cation, phosphate is a major intracellular anion. Therefore, when blood is drawn for serum inorganic phosphate measurement, hemolysis of the specimen must be avoided. Also, serum should be removed from the clot as soon after collection as possible to avoid leakage of phosphate into the serum. Both of these situations would contribute to falsely increased serum phosphate levels. Conversely, serum phosphate levels will be depressed following meals, during the menstrual period, and during intravenous glucose and fructose therapy.

311.

B. Copper is found in the plasma mainly in two forms: a minor fraction loosely bound to albumin and the majority, representing about 80–95%, firmly bound to the enzyme ceruloplasmin, an α_2-globulin, which is important in the oxidation of iron from the ferrous to the ferric state. Copper is also an essential constituent of a variety of other enzymes found in erythrocytes and in other sites throughout the body. The major clinical usefulness of determining serum copper or ceruloplasmin levels is that the decreased level of both is associated with Wilson disease. Decreased levels of copper are also found in protein malnutrition and malabsorption and in nephrosis.

312.

A. Transferrin is a glycoprotein that reversibly binds serum iron that is not combined with other proteins such as hemoglobin and ferritin. Transferrin concentration in serum is rarely determined directly but, rather, in terms of the serum iron content after saturation with iron. This is the total iron-binding capacity (TIBC). The percent saturation of transferrin is determined by dividing the serum iron level by the serum TIBC and expressing this value as a percentage. Normally in adults the percent saturation of transferrin is in the range of 20–50%, whereas in iron-deficiency anemia, the saturation is expected to be less than 15%. In iron-deficiency anemia complicated by other disorders that either increase serum iron concentration or decrease the TIBC, the percent saturation may remain within the reference range.

313.

C. In adults the total body iron content averages 3–4 g. The majority of this iron is found in the active pool as an essential constituent of hemoglobin, with a much lesser amount being an integral component of myoglobin and a number of enzymes. Approximately 25% of the body iron is found in inactive storage forms. The major storage form of iron is ferritin, with a lesser amount being stored as hemosiderin. Ferritin may be found in most body cells but especially in reticuloendothelial cells of the liver, spleen, and bone marrow.

314.

D. In cases of iron-deficiency anemia uncomplicated by other diseases, serum ferritin levels correlate well with the evidence of iron deficiency obtained by marrow examination for stainable iron. This indicates that ferritin is released into the serum in direct proportion to the amount stored in tissues. In iron deficiency, serum ferritin levels fall early in the disease process. However, in certain disorders there is a disproportionate increase in serum ferritin in relation to iron stores. Examples include chronic infections, chronic inflammation, malignancies, and liver disease. For individuals who have these chronic disorders or iron deficiency, it is common for their serum ferritin levels to appear normal.

315.

B. Serum iron concentrations are most often determined by the colorimetric reaction with ferrozine, bathophenanthroline, or tripyridyltriazine. The same reagent is usually used in the determination of serum TIBC by saturating the transferrin in the serum with an excess of iron, removing any unbound iron, and measuring the iron bound to transferrin. This measurement of TIBC provides a measure of transferrin concentration. Several magnesium methods require the precipitation of magnesium as part of the analysis, and 8-hydroxyquinoline effectively precipitates magnesium.

316.

A. Transferrin is the iron transport protein in serum and is normally saturated with iron to the extent of approximately 20–50%. An increased percent saturation of transferrin is expected in patients with hemochromatosis, an iron overload disease, and iron poisoning. The increased saturation is due to the increased iron concentration in the serum. In patients with chronic infections and malignancies, there is impairment of iron release from body storage sites, leading to a decreased percent saturation of transferrin. In myocardial infarction the serum iron levels are depressed, but the TIBC levels are normal. Iron-deficiency anemia because of poor absorption, poor diet, or chronic loss results in decreased serum iron, increased transferrin, and decreased percent saturation of transferrin in most cases.

317.

C. In order to differentiate among diseases, it is necessary to perform several laboratory determinations to properly assess iron metabolism. In iron-deficiency anemia, the serum iron is decreased whereas the TIBC is increased. Thus it follows that the transferrin saturation is decreased. The serum ferritin level, which represents stored body iron, is depressed, and the free erythrocyte protoporphyrin (FEP) level is increased. FEP is not a specific test for iron-deficiency anemia, but it can function as a screening test.

318.

A. A low ionized serum magnesium level is characteristic of a magnesium deficiency tetany. The serum magnesium level usually ranges between 0.15 and 0.5 mmol/L when tetany occurs. In addition, the serum calcium level and blood pH are normal, whereas the serum potassium level is decreased. This type of tetany is treated with $MgSO_4$ to increase the level of serum magnesium, thus alleviating the tetany and convulsions that accompany this disorder.

319.

A. Magnesium measurements are commonly done spectrophotometrically using reagent systems such as calmagite, methylthymol blue, and chlorophosphonazo III. Calcium will interfere and is eliminated by complexing with a chelator that binds calcium and not magnesium. Atomic absorption is a specific and sensitive method for analysis of magnesium, with the only significant interference being phosphate ions, which are removed by complexing with a lanthanum salt.

320.

D. Plasma lactate concentrations are increased in cases of lactic acidosis. The accumulation of lactate in the blood results from any mechanism that produces oxygen deprivation of tissues and, thereby, anaerobic metabolism. Lactate concentrations in whole blood are extremely unstable because of the rapid production and release of lactate by erythrocytes as a result of glycolysis. One method of stabilizing blood lactate levels in specimen collection is to add an enzyme inhibitor such as fluoride or iodoacetate to the collection tubes. Heparin, ethylenediaminetetra-acetic acid (EDTA), and oxalate will act as anticoagulants but will not prevent glycolysis in the blood sample.

321.

C. Measuring the concentration of chloride in sweat is a commonly used diagnostic procedure for determining the disorder of cystic fibrosis (CF). The majority of patients with CF will present with increased concentrations of sodium and chloride in their sweat. Generally, children with CF will manifest sweat chloride levels that are two to five times the reference interval. In sweat testing, sweat production is stimulated by iontophoresis with pilocarpine. Then the sweat is either collected and analyzed for chloride or an ion-selective electrode is applied to the skin surface to quantify chloride. It has been established that the gene abnormality causing CF is located on chromosome 7.

322.

A. Colligative properties of a solution are those properties that depend only on the number of particles in solution, not on the nature of the particles. The colligative properties are boiling point, freezing point, osmotic pressure, and vapor pressure. Terms used to describe the concentration of particles in solution are "osmole" (the number of particles, 6.0224×10^{23}, that lowers the freezing point 1.86°C) and "osmolal" (a concentration of 1 Osm of solute per kilogram of water). One mole of an un-ionized solute dissolved in 1 kg of water lowers the freezing point 1.86°C. Thus it is an osmolal solution. For un-ionized substances such as glucose, 1 mol equals 1 Osm. For substances that ionize, such as sodium chloride, wherein each molecule in solution becomes two ions and thus two particles, 1 mol of sodium chloride theoretically equals 2 Osm. In reality, however, this is not always the case; an osmotic activity coefficient factor is used to correct for the deviation. In practice, three types of osmometers are available. They are the freezing point, vapor pressure, and colloid osmotic pressure osmometers.

323.

D. The freezing point of an aqueous solution is lowered 1.86°C for every osmole of dissolved particles per kilogram of water. These particles may be ions (e.g., Na^+ and Cl^-), or undissociated molecules such as glucose. The freezing point osmometer is an instrument designed to measure the freezing point of solutions. It uses a thermistor that is capable of measuring very small changes in temperature.

324.

D. When the osmolality has been both measured in the laboratory and calculated, the osmolal gap may then be determined by subtracting the calculated osmolality from the measured. Plasma osmolality may be calculated when the plasma sodium, glucose, and urea nitrogen values are known. The equation for calculating osmolality expresses Na^+, glucose, and urea nitrogen in mmol/L (SI units). To convert glucose and urea nitrogen from mg/dL to mmol/L, the conversion factors 0.056 and 0.36 are used, respectively. For sodium, the factor 2 is used to count the cation (sodium) once and its corresponding anion once. Because glucose and urea nitrogen are undissociated molecules, they are each counted once. Use the following equation:

$$\begin{aligned}&\text{Calculated osmolality (mOsm/kg)}\\&= 2.0\ Na^+(\text{mmol/L}) + \text{Glucose (mmol/L)}\\&\qquad + \text{Urea nitrogen (mmol/L)}\\&= 2.0\ (142\ \text{mmol/L}) + (0.056 \times 130\ \text{mg/dL})\\&\qquad + (0.36 \times 18\ \text{mg/dL})\\&284 + 7.3 + 6.5 = 298\ \text{mOsm/kg}\end{aligned}$$

325.

C. The colloid osmotic pressure (COP) osmometer is composed of a semipermeable membrane that separates two chambers, a mercury manometer, a pressure transducer, and a meter. When a serum sample is introduced into the sample chamber, saline solution from the reference chamber moves across the membrane by osmosis. This causes the development of a negative pressure on the saline side that is equivalent to the COP, which represents the amount of protein in the serum sample. COP osmometers measure the serum protein contribution to the total osmolality in terms of millimeters of mercury. COP levels are helpful in monitoring intravenous fluid therapy.

Acid-Base Metabolism

326.

A. Because of its high concentration in blood, the bicarbonate/carbonic acid pair is the most important buffer system in the blood. This buffer system is also effective in the lungs and in the kidneys in helping to regulate body pH. The other buffers that also function to help maintain body pH are the phosphate, protein, and hemoglobin buffer systems. The acetate buffer system is not used by the body to regulate pH.

327.

C. PCO_2 is an indicator of carbonic acid (H_2CO_3). The PCO_2 millimeters of mercury value (mm Hg) multiplied by the constant 0.03 equals the millimoles per liter (mmol/L) concentration of H_2CO_3 ($PCO_2 \times 0.03 = H_2CO_3$). PCO_2 can be measured using a pH/blood gas analyzer.

328.

A. The concentration of total CO_2 ($ctCO_2$) or carbon dioxide content is a measure of the concentration of bicarbonate, carbonate, carbamino compounds, carbonic acid, and dissolved carbon dioxide gas (PCO_2) in the plasma. Bicarbonate makes up approximately 95% of the total CO_2 content, but most laboratories are not equipped to directly measure bicarbonate. Therefore, total CO_2 is generally quantified. The bicarbonate concentration may be estimated by subtracting the H_2CO_3 concentration (measured in terms of PCO_2 and converted to H_2CO_3) from the total CO_2 concentration.

329.

B. The most important buffer pair in the plasma is bicarbonate with carbonic acid. Use of the Henderson-Hasselbalch equation

$$pH = pK' + \log\frac{[salt]}{[acid]}$$

shows that the pH changes with the ratio of salt to acid—that is, bicarbonate to carbonic acid—because pK′ is a constant. For this buffer pair, apparent pK′ = 6.1. When the ratio of the concentrations of bicarbonate to carbonic acid is 20:1 (log of 20 = 1.3), the pH is 7.4; that is,

$$pH = 6.1 + \log 20$$
$$7.4 = 6.1 + 1.3$$

The carbonic acid designation represents both the undissociated carbonic acid and the physically dissolved carbon dioxide found in the blood. Because the concentration of the undissociated carbonic acid is negligible compared to the concentration of physically dissolved carbon dioxide, the expression for carbonic acid concentration is usually written ($PCO_2 \times 0.03$).

330.

D. The acid-base equilibrium of the blood is expressed by the Henderson-Hasselbalch equation:

$$pH = pK' + \log\frac{c\mathrm{HCO_3^-}}{(PCO_2 \times 0.03)}$$

In this buffer pair, pK′ = 6.1. Normally, the ratio of the concentration of bicarbonate ions $c\mathrm{HCO_3^-}$ to the concentration of carbonic acid expressed as ($PCO_2 \times 0.03$) in the plasma is 20:1. The bicarbonate component of the equation is considered to be the "metabolic" component, controlled by the kidneys. The carbonic acid component is considered the "respiratory" component, controlled by the lungs. An excess of bicarbonate without a change in PCO_2 will increase the ratio of bicarbonate to carbonic acid. Therefore, the pH will increase; that is, the plasma becomes more alkaline.

331.

C. The normal ratio of bicarbonate ions to dissolved carbon dioxide is 20:1 and pH = 6.1 + log 20/1. An excess of dissolved CO_2 (e.g., increase in PCO_2) will increase the denominator in the equation or decrease the ratio of bicarbonate ions to dissolved CO_2. The pH will decrease; that is, the plasma becomes more acidic. The amount of dissolved CO_2 (PCO_2) in the blood is related to respiration. Hence, this condition is termed "respiratory acidosis."

332.

B. It is possible to use arterial, venous, or capillary blood for blood gas analysis. The specimen of choice for determining pulmonary dysfunction in adults is arterial blood. Analysis of arterial blood is the best indicator of pulmonary function, the capacity of the lungs to exchange carbon dioxide for oxygen. PO_2 and PCO_2 measurements from capillary blood are usually confined to infant sampling, and they are dependent on the patient preparation and sampling site. Venous blood should not be used for blood gas studies involving pulmonary problems because venous blood gas values also reflect metabolic processes. Furthermore, the reference range for PO_2 in venous blood varies drastically from arterial blood. Urine cannot be used to determine the acid/base status of a patient.

333.

B. Heparin is the best anticoagulant to use in drawing blood for blood gas analyses because it does not affect the value of the blood pH. This is also critical to PO_2 measurements because alterations in blood pH will cause concomitant changes in PO_2 values. Several heparin salts are available for use as anticoagulants. Sodium heparinate, 1000 U/mL, is commonly used. Ammonium heparinate may be substituted for the sodium salt when it is necessary to perform additional testing, such as electrolyte analysis, on the blood gas sample.

334.

A. When a blood specimen is drawn for gas analysis, it is important to avoid exposure of the specimen to air because of the differences in the partial pressures of carbon dioxide and oxygen in air and in blood. The PCO_2 in blood is much greater than the PCO_2 in air. Hence on exposure of blood to air, the total CO_2 and the PCO_2 both decrease, causing an increase in pH. Similarly, the PO_2 of air is much greater than that of blood, thus, the blood PO_2 increases on exposure to air.

335.

C. Glycolysis and other oxidative metabolic processes will continue *in vitro* by red blood cells when a whole blood specimen is left standing at room temperature. Oxygen is consumed during these processes, resulting in a decrease in PO_2 levels. A decrease of 3–12 mm Hg/hr at 37°C has been observed for blood specimens exhibiting normal PO_2 ranges. This rate of decrease is accelerated with elevated PO_2 levels. Additionally, carbon dioxide is produced as a result of continued metabolism. An increase in PCO_2 levels of approximately 5 mm Hg/hr at 37°C has been demonstrated. The increased production of carbonic acid and lactic acid during glycolysis contributes to the decrease in blood pH.

336.

D. The solubility coefficient of CO_2 gas (dissolved CO_2) in normal blood plasma at 37°C is 0.03 mmol/L/mm Hg. The concentration of dissolved CO_2 found in plasma is calculated by multiplying the PCO_2 blood level by the solubility coefficient (0.03). The predominant components of total CO_2 are bicarbonate (95%) and carbonic acid (5%). The bicarbonate ion concentration in millimoles per liter can be calculated by subtracting the product of (0.03 mmol/L/mm Hg × PCO_2 mm Hg), which represents carbonic acid, from the total CO_2 concentration (millimoles per liter).

337.

C. The red blood cell membrane is permeable to both bicarbonate and chloride ions. Chloride ions participate in buffering the blood by diffusing out of or into the red blood cells to compensate for the ionic change that occurs when bicarbonate enters or leaves the red blood cell. This is called the chloride shift.

338.

B. In the diabetic patient, diabetic ketoacidosis is one of the complications that may require emergency therapy. Blood glucose levels are usually in the range of 500–700 mg/dL but may be higher. The result is severe glycosuria that produces an osmotic diuresis, leading to loss of water and depletion of body electrolytes. Lipolysis is accelerated as a result of insulin deficiency. The free fatty acids produced are metabolized to acetyl-coenzyme A units, which are converted in the liver to ketone bodies. Hydrogen ions are produced with ketone bodies (other than acetone), contributing to a decrease in blood pH. Ketoacids are also excreted in the urine, causing a decrease in urinary pH.

339.

D. One of the primary reasons for metabolic alkalosis, especially in infants, is vomiting. Hydrogen ions are lost in the vomit, and the body reacts to replace them in the stomach. Consequently, hydrogen is lost from the plasma. This loss of hydrogen is due to a metabolic as opposed to a respiratory reason. Salicylate poisoning, uncontrolled diabetes mellitus, and renal failure all lead to metabolic acidosis either through an overproduction of ketone bodies, such as acetoacetic acid and beta-hydroxybutyric acid, or because of a reduced excretion of acid by the kidneys.

340.

D. Laboratory results from arterial blood gas studies in partially compensated respiratory alkalosis are as follows: pH slightly increased, PCO_2 decreased, HCO_3^- decreased, and total CO_2 decreased. Respiratory alkalosis is a disturbance in acid-base balance that is caused by hyperventilation associated with such conditions as fever, hysteria, and hypoxia. Respiratory alkalosis is characterized by a primary deficiency in physically dissolved CO_2 (decreased PCO_2). This decrease in the level of PCO_2 is due to hyperventilation, causing the accelerated loss of CO_2 by the lungs. This loss of CO_2 alters the normal 20:1 ratio of $cHCO_3^-/PCO_2$, causing an increase in the blood pH level. In respiratory alkalosis, because the initial defect is in the lungs, the kidneys respond as the major compensatory system. Ammonia production in the kidneys is decreased, Na^+–H^+ exchange is decreased with the retention of H^+, and bicarbonate reabsorption is decreased. By decreasing the bicarbonate reabsorption into the bloodstream, the kidneys attempt to reestablish the 20:1 ratio and normal blood pH. In a partially compensated state, as the blood bicarbonate level decreases, the blood pH begins to return toward normal but continues to be slightly alkaline. In a fully compensated state the blood pH is normal.

341.

C. Respiratory acidosis is a disturbance in acid-base balance that is caused by the retention of CO_2 by the lungs. This imbalance is associated with such conditions as bronchopneumonia, pulmonary emphysema, pulmonary fibrosis, and cardiac insufficiency. Respiratory acidosis is characterized by a primary excess in physically dissolved CO_2, which is quantified by measuring the blood PCO_2 level. The primary problem leading to an increase in the PCO_2 level is hypoventilation. This retention of CO_2 alters the normal 20:1 ratio of $cHCO_3^-/PCO_2$, causing a decrease in blood pH level. In respiratory acidosis, because the initial defect is associated with the lungs, the kidneys respond as the major compensatory system. The production of ammonia, the exchange of Na^+ for H^+ with the excretion of H^+, and the reabsorption of bicarbonate are all increased in the kidneys to compensate for the malfunction of the lungs. In cases where the defect is not within the respiratory center, the excess of PCO_2 in the blood can actually have a stimulatory effect on the center, causing an increase in the respiration rate. Thus compensation can also occur through CO_2 elimination by the lungs.

342.

A. There is a wide variety of conditions that will cause a shift of the dissociation curve of oxyhemoglobin to the left or to the right. A shift to the left will mean an increase in the affinity of hemoglobin for oxygen. Because of this increased affinity, there is also less oxygen delivered to the tissue for a given percent saturation of hemoglobin. When the curve is shifted to the right, there is a decrease in the affinity of hemoglobin for oxygen. Hence there is increased oxygen delivered to tissues for a given hemoglobin oxygen saturation. Oxyhemoglobin is a stronger acid than deoxyhemoglobin. Both exist in equilibrium in the blood. Increased hydrogen ion concentration shifts the equilibrium toward the deoxygenated form. This shift results in increased oxygen delivery to the tissue. The higher the concentration of 2,3-bisphosphoglycerate in the cell, the greater is the displacement of oxygen, thus facilitating the release of oxygen at the tissue level. Increased PCO_2 and increased temperature will also have this same effect.

343.

A. Carbonic anhydrase (CA) is an enzyme found in red blood cells that catalyzes the reversible hydration of CO_2 to bicarbonate and a proton:

$$H_2O + CO_2 \overset{CA}{\rightleftharpoons} HHCO_3$$

The proton, in turn, is buffered by the histidine portion of the hemoglobin molecule that activates the release of oxygen. It is at this point that oxyhemoglobin is converted to deoxyhemoglobin. In the alveoli of the lungs, CA catalyzes the conversion of H_2CO_3 to CO_2 and H_2O. The CO_2 is then exhaled. Carbonic anhydrase is an intracellular enzyme of erythrocytes and renal tubular cells, and it is not found normally in any significant concentration in the plasma. It is not associated with the oxygen dissociation curve.

344.

B. Base excess is a measure of the nonrespiratory buffers of the blood. They are hemoglobin, serum protein, phosphate, and bicarbonate. Therefore, base excess reflects an abnormality in the buffer base concentration. Bicarbonate has the greatest influence on base excess, which is an indicator of metabolic function. The normal range for base excess is ±2.5 mmol/L. A quick estimation of base excess is to subtract the average "normal" reference bicarbonate level set by the laboratory from the measured bicarbonate level (e.g., if laboratory reference bicarbonate = 25 and patient's bicarbonate = 30, then base excess = (30 − 25) = +5; if patient's bicarbonate = 20, then base excess = (20 − 25) = −5). As demonstrated, a positive base excess is associated with metabolic alkalosis, and a negative base excess is associated with metabolic acidosis.

345.

C. The acid-base equilibrium of the blood is expressed by the Henderson-Hasselbalch equation:

$$pH = pK' + \log \frac{cHCO_3^-}{(PCO_2 \times 0.03)}$$

For the stated problem, convert PCO_2 in mm Hg to dissolved CO_2, multiplying by the solubility coefficient of CO_2 gas: 44 mm Hg × 0.03 mmol/L/mm Hg = 1.32 mmol/L. Next, determine the bicarbonate concentration by finding the difference between the total CO_2 and dissolved CO_2 concentrations: 29 mmol/L − 1.32 mmol/L = 27.68 mmol/L. pK′ for the bicarbonate buffer system is 6.1. Therefore,

$$pH = 6.1 + \log \frac{27.68}{1.32}$$

$$pH = 6.1 + \log 20.97$$

$$pH = 6.1 + \log 21$$

$$pH = 6.1 + 1.32$$

$$pH = 7.42$$

346–349.

In evaluating acid-base balance, the pH, PCO_2, and total CO_2 of an arterial blood specimen are measured. The reference values of arterial whole blood at 37°C for adults are as follows:

$$\begin{aligned} pH &= 7.35–7.45 \\ PCO_2 &= 35–45 \text{ mm Hg} \\ cHCO_3^- &= 22–26 \text{ mmol/L} \\ ctCO_2 &= 23–27 \text{ mmol/L} \\ PO_2 &= 80–110 \text{ mm Hg} \end{aligned}$$

Acid-base disturbances can be characterized into four basic disorders: metabolic alkalosis, metabolic acidosis, respiratory alkalosis, and respiratory acidosis.

$$pH = pK' + \log\frac{cHCO_3^-}{H_2CO_3}$$

or

$$pH = pK' + \log\frac{cHCO_3^-}{(PCO_2 \times 0.03)}$$

Normally, the average ratio of bicarbonate to the concentration of carbonic acid is 20:1, resulting in a blood pH of 7.4. The $cHCO_3^-$ is represented in the measurement of total CO_2 value because 95% of the total CO_2 is HCO_3^-. The concentration of carbonic acid is calculated by multiplying the PCO_2 value by 0.03 (the solubility coefficient of CO_2 gas). The bicarbonate (base) represents the renal component of the acid-base balance. It is related to metabolic function. The dissolved carbon dioxide, measured as PCO_2, represents the respiration component and is related to respiratory function. Thus respiratory acidosis is characterized by an increase in blood PCO_2, whereas respiratory alkalosis is characterized by a decrease of blood PCO_2. Metabolic acidosis is characterized by a decrease in the blood bicarbonate levels, whereas metabolic alkalosis is related to an increase in blood bicarbonate levels. In acid-base disorders, the compensatory changes occur in the component that is not the original cause of the imbalance if compensation can occur. Thus in an acid-base imbalance of respiratory origin, the kidneys exert the major corrective action. In an acid-base imbalance of metabolic origin, the lungs exert the major corrective action. Sometimes a "mixed" or "double" problem of acidosis and alkalosis may exist due to more than one pathological process (e.g., a diabetic with asthma where both the respiratory and metabolic components indicate acidosis). If neither the respiratory nor the metabolic components indicate the condition of the patient (e.g., acidosis or alkalosis), then, most likely, there is something wrong with one or more of the blood gas results. In approaching acid-base problems, one should first key on the pH to determine the general condition (acidosis or alkalosis), then ask what is causing it—for example, a change in bicarbonate or PCO_2—to determine if the problem is metabolic or respiratory, and finally look at the remaining component to see if there is compensation bringing the pH closer to 7.4. If there is no movement in the remaining component from the reference value, then there is no compensation or uncompensation.

346.

A. In this case the pH is increased indicating alkalosis. HCO_3^- is increased, which means it is a metabolic problem. The PCO_2 is also increased, which indicates that the lungs are trying to compensate by retaining PCO_2 thus bringing the pH closer to 7.4.

347.

B. Here the pH is decreased indicating acidosis. The PCO_2 is increased, which indicates that the problem is respiratory in nature. The HCO_3^- is unchanged from the reference range, which indicates that there is no compensation; thus the patient has uncompensated respiratory acidosis.

348.

C. The pH clearly indicates acidosis. Both the metabolic (decreased HCO_3^-) and respiratory (increased PCO_2) components, however, indicate acidosis. There is no compensation seen in the results. Thus the patient has a double or mixed problem of acidosis.

349.

D. Here the pH and case information indicate alkalosis, but both the metabolic (decreased HCO_3^-) and respiratory (increased PCO_2) components indicate acidosis. Most likely there is a problem/error in one or more of the measurements.

Endocrinology

350.

C. The hypothalamus produces releasing factors or hormones that affect the release and synthesis of anterior pituitary hormones. The releasing hormones could have a stimulatory effect, as in the case of luteinizing hormone-releasing hormone (LH-RH), or an inhibitory effect, as in the case of prolactin-inhibiting factor (PIF). The posterior lobe of the pituitary acts only as a storage area for vasopressin and oxytocin, which are manufactured in the hypothalamus. The posterior lobe of the pituitary gland does not affect any feedback control on the anterior lobe. The intermediate lobe secretes beta-melaninophore-stimulating hormone, which acts on the skin. It also does not affect any control over the anterior lobe. The adrenal medulla secretes catecholamines, which are not involved in any feedback mechanism to the pituitary gland.

351.

B. Adrenocorticotropic hormone (ACTH) stimulates the adrenal cortex to secrete cortisol and, to a certain extent, aldosterone. However, aldosterone is also regulated by sodium and potassium levels and, more importantly, by the renin-angiotensin system. Cortisol alone has an inhibitory effect or a negative feedback relationship to ACTH secretion by the pituitary. A low level of cortisol stimulates the hypothalamus to secrete corticotropin-releasing hormone (CRH), which in turn stimulates release of ACTH from the pituitary gland and causes the adrenal cortex to secrete more cortisol. Elevated levels of cortisol reverse this process. ACTH secretion is not inhibited by estrogen or progesterone levels.

352.

A. The corticosteroids, produced by the adrenal cortex, may be classified as glucocorticoids or mineralocorticoids. Cortisol is the primary glucocorticoid, and aldosterone is the primary mineralocorticoid. Aldosterone functions as a regulator of salt and water metabolism. Aldosterone promotes water retention and sodium resorption with potassium loss in the distal convoluted tubules of the kidney.

353.

B. Renin is a proteolytic enzyme secreted by the juxtaglomerular cells of the kidneys. In the blood, renin acts on renin substrate (angiotensinogen) to produce angiotensin I. An angiotensin-converting enzyme secreted by endothelial cells then converts angiotensin I to angiotensin II. It is the latter that is responsible for the vasoconstrictive action of renin release. Angiotensin III is a product of aminopeptidase on angiotensin II, and the action of angiotensin II and III is directed at modulating aldosterone secretion. Plasma renin activity, determined by immunoassay, is assessed by quantifying the amount of angiotensin I produced by the action of renin on angiotensinogen using an initial kinetic assay. In addition, renin can be measured directly by an immunometric-mass assay that utilizes a monoclonal antibody.

354.

A. A low-salt diet, upright position, and diuretics cause a decrease in effective plasma volume. This decrease stimulates the renin-angiotensin system, which increases aldosterone secretion. Aldosterone promotes sodium retention and potassium loss.

355.

B. The hypothalamus, which secretes CRH, is sensitive not only to cortisol levels and stress but also to sleep-wake patterns. Thus plasma ACTH and cortisol levels exhibit diurnal variation or circadian rhythm. Cortisol secretion peaks at the time of awakening between 6 A.M. and 8 A.M. and then declines to the lowest level between early evening and midnight. After midnight the level again begins to increase. Specimens should be taken at 8 A.M. and 8 P.M. The evening cortisol level should be at least 50% lower than the morning result. In 90% of patients with Cushing syndrome there is no diurnal variation. However, absence of the normal drop in the evening cortisol level is not specific for Cushing syndrome. Other conditions, such as ectopic ACTH syndrome, blindness, hypothalamic tumors, obesity, acute alcoholism, and various drugs, alter normal circadian rhythm in cortisol secretion. To confirm Cushing syndrome, a dexamethasone suppression test may be performed.

356.

D. For differentiation of primary and secondary adrenal dysfunction, stimulation or suppression tests that depend on the feedback mechanism between cortisol and ACTH are performed. In the ACTH stimulation test, a patient with a low baseline serum cortisol level is given ACTH. The level of cortisol will increase slightly if the problem lies with the anterior pituitary gland, thus secondary adrenal insufficiency. This increase will be less than normal and may be somewhat delayed due to atrophy of the adrenal cortex as a result of the primary pituitary dysfunction. If the serum cortisol level does not change from baseline, the dysfunction is with the adrenal cortex, thus primary adrenal insufficiency.

357.

C. Only very small quantities, normally less than 2%, of the total adrenal secretion of cortisol appear in the urine as free cortisol. The majority of cortisol is either metabolized in various tissues or conjugated in the liver and excreted. It is only the serum unconjugated cortisol not bound to corticotropin binding globulin (CBG) or the conjugated cortisol that can be cleared by glomerular filtration in the kidney. Therefore, the measurement of free cortisol in the urine is a sensitive reflection of the amount of unbound cortisol in the serum. It is not a reflection of the amount of conjugated cortisol or the serum total cortisol but, rather, only the increased cortisol production that is not accompanied by an increase in serum levels of CBG.

358.

C. The probable diagnosis is Cushing syndrome caused by adrenocortical carcinoma. In adrenocortical carcinoma, the urinary free cortisol and the serum cortisol levels would be elevated and the plasma ACTH level would be decreased. The carcinoma produces excess cortisol that, because of the feedback loop, turns off pituitary production of ACTH. Neither the low-dose dexamethasone suppression test nor the high-dose test is able to suppress cortisol production. Because dexamethasone is a cortisol analogue, it would normally suppress ACTH and cortisol levels in a healthy individual. All these data support primary adrenal dysfunction caused by an adrenal carcinoma. If the elevated cortisol level was due to a pituitary adenoma or ectopic ACTH lung cancer, the ACTH level would also be increased. Addison disease is caused by hypofunction of the adrenal cortex.

359.

B. The adrenogenital syndrome, congenital adrenal hyperplasia, is due to a deficiency in specific enzymes needed for the synthesis of cortisol and aldosterone. Because cortisol production is blocked, the pituitary increases its secretion of adrenocorticotropic hormone (ACTH), causing adrenal hyperplasia and hypersecretion of cortisol precursors. There are eight recognized types of inherited enzyme defects in cortisol biosynthesis. The most common type of defect is the lack of 21-hydroxylase, occurring in 95% of the cases. Conversion of 17α-hydroxyprogesterone to 11-deoxycortisol is impaired, causing accumulation of 17α-hydroxyprogesterone, which is metabolized to pregnanetriol. An increased plasma 17α-hydroxyprogesterone level is diagnostic and can be determined by radioimmunoassay. Determinations of serum testosterone and urinary pregnanetriol elevations are also diagnostic of this disorder. Virilization takes place in this syndrome because cortisol precursors are shunted to produce weak androgens [e.g., dehydroepiandrosterone (DHEA) and androstenedione]. These androgens are converted peripherally to testosterone in large-enough amounts to create this condition. The second most common defect is 11β-hydroxylase deficiency with an accumulation of 11-deoxycortisol. 3β-Hydroxysteroid dehydrogenase-isomerase deficiency and C-17,20-lyase/17α-hydroxylase deficiency are examples of other enzyme defects seen in this disorder. A testicular or adrenal tumor may cause symptoms similar to this syndrome; however, these tumors would be acquired in contrast to congenital disorders.

360.

D. Testosterone is the most potent of the body's androgens. One of the major functions of the testes is to produce testosterone. It is metabolized to the 17-ketosteroids, etiocholanolone and androsterone, but testosterone is not itself a 17-ketosteroid. The 17-ketosteroids, dehydroepiandrosterone (DHEA), androsterone, and androstenedione, all have androgenic properties but are much weaker than testosterone.

361.

B. The pituitary gland produces protein hormones such as adrenocorticotropic hormone, thyroid-stimulating hormone, follicle-stimulating hormone, growth hormone, and prolactin. Steroid hormones include C_{21} corticosteroids and progesterone, C_{19} androgens, and C_{18} estrogens. The mineralo- and gluco-corticosteroids are secreted only by the adrenal glands, but the other steroids listed are secreted by the ovaries, testes, adrenal glands, and placenta to a varying extent, depending on the individual's sex.

362.

C. 17β-Estradiol (E_2) is the most potent estrogen. 17β-Estradiol is considered to be the true ovarian hormone because it is secreted almost entirely by the ovaries. In contrast, estrone (E_1) is produced from circulating C_{19} neutral steroids (e.g., androstenedione) and is also synthesized from 17β-estradiol. Estriol (E_3) is derived almost exclusively from 17β-estradiol and has little clinical significance except in pregnancy. The measurement of 17β-estradiol is used to evaluate ovarian function.

363.

C. In pregnant women the level of human chorionic gonadotropin (hCG) is highest during the first trimester, then it stabilizes to a lower level during the rest of the pregnancy. In the first trimester, the level of pregnanediol is slightly higher than that found in nonpregnant women during the luteal phase of the menstrual cycle. As pregnancy progresses, the placenta secretes more progesterone, which peaks midway into the third trimester and then levels off. It should be noted that pregnanediol is a biologically inactive metabolite of progesterone that is sometimes measured in urine. After the second month of pregnancy, estriol levels steadily increase as the placenta takes over estrogen production.

364.

C. The triple test for Down syndrome includes quantification of α_1-fetoprotein (AFP), unconjugated estriol (uE_3), and human chorionic gonadotropin (hCG) in the maternal serum. These measurements should be done between 16 and 18 weeks gestation, and they are useful in detecting neural tube defects and Down syndrome. In Down syndrome, the AFP and uE_3 levels are low, whereas the hCG level is elevated. These test results are related to gestational age and are expressed as a multiple of the median (MoM), meaning the maternal serum result is divided by the median result of the corresponding gestational population.

365.

A. Progesterone production can be monitored by measuring plasma progesterone or urinary pregnanediol, the major metabolite of progesterone. In the follicular stage of the menstrual cycle, only a small amount of progesterone is secreted. In the luteal stage, or the time from ovulation to menstruation, progesterone levels rapidly increase. Hence, serial assays of plasma progesterone or urinary pregnanediol can be used to identify the time of ovulation. If pregnancy does not occur, progesterone quickly decreases approximately 24 hours before menstruation. If there is no ovulation, then there is no corpus luteum formation and no cyclic rise in progesterone levels.

366.

D. Luteinizing hormone (LH) is secreted only by the anterior pituitary. A protein hormone, human chorionic gonadotropin (hCG), appears soon after conception and is thus used for early detection of pregnancy. Human placental lactogen (HPL), also a protein hormone, is produced only by the placenta and is measurable between the seventh and ninth weeks. HPL steadily increases throughout pregnancy and peaks near term. Analysis of HPL for placental dysfunction has been successful; however, it is not widely used for this purpose. During pregnancy the placenta is the main source of estrogen and progesterone. Both hormones are needed for the maintenance of pregnancy.

367.

D. The formation of estriol during pregnancy involves mainly the fetoplacental unit. Dehydroepiandrosterone sulfate (DHEA-S) and its 16α-hydroxy-DHEA-S derivative are formed mainly by the fetal adrenal glands and to a lesser degree by the liver. The fetus possesses 16α-hydroxylase activity, which is needed to convert dehydroepiandrosterone sulfate (DHEA-S) to 16α-hydroxy-DHEA-S. The 16α-hydroxy-DHEA-S compound is metabolized by the placenta to estriol. The placenta lacks certain enzymes needed for the conversion of simple precursors such as acetate, cholesterol, and progesterone to estrogens. Thus, the placenta must rely on immediate precursors produced in the fetus. In the case of estriol, the placenta utilizes the 16α-hydroxy-DHEA-S precursor made in the adrenal glands of the fetus. The latter compound crosses into the placenta, which takes over with the necessary enzymes to complete the synthesis of estriol. This estriol produced in the placenta is rapidly reflected in the maternal plasma and far exceeds maternal synthesis of estriol. Thus measurement of estriol in the maternal blood or urine is a sensitive indicator of the integrity of the fetoplacental unit. A defect in either the fetus or the placenta will be reflected by a decrease in estriol production.

368.

D. The concentration of estriol in maternal plasma or in a 24-hour sample of maternal urine is often used as an indicator of fetal distress or placental failure. A single value of either serum or urine estriol has relatively little value unless it can be related accurately to the gestational week. When sequential estriol determinations are made during pregnancy, a pattern of stable or steadily falling values may indicate a problem pregnancy. For serum or urine estriols, any individual value that is 30–50% less than the previous value or the average of the previous 3 days' values is significant.

369.

D. Acetate, cholesterol, progesterone, and the male sex hormones testosterone and androstenedione all serve as precursors for the synthesis of estrogens. The major pathway for conversion of testosterone to estradiol is in the ovaries. The major pathway for conversion of androstenedione to estrone is outside the ovaries.

370.

C. During the menstrual cycle, follicle-stimulating hormone (FSH) levels decrease in the later part of the follicular stage. Luteinizing hormone (LH) gradually increases during the follicular stage. At midcycle, both FSH and LH levels spike. Following this spike, in the luteal stage or second half of the menstrual cycle, FSH and LH levels gradually decrease. In postmenopausal women the ovaries stop secreting estrogens. In response the gonadotropins, FSH and LH, rise to their highest levels. The reason is the feedback system between estrogen secretion by the gonads and the secretion of releasing factors by the hypothalamus; a decreased estrogen level causes increased secretion of FSH-releasing factor and LH-releasing factor.

371.

C. Ectopic hormones are hormonal substances produced by benign and malignant tumors derived from tissues that do not normally secrete those hormones. Examples of ectopic hormone production would be ACTH production by oat cell carcinoma of the lung and growth hormone production by bronchogenic carcinomas of the lung. Cortisol and growth hormone are normally secreted by the adrenal gland and anterior pituitary gland, respectively. Ectopic hormones are not in all cases chemically identical to the native hormone but may be similar enough to cross-react in immunoassay methods for the native hormone.

372.

A. At the cellular level, the site of action of the peptide and catecholamine hormones is different from that of the steroid and thyroid hormones. The peptide and catecholamine hormones bring about their effects by combining with receptors on or in the cell membranes of the target cells. In some cases, this binding to the membrane results in activation of adenylate cyclase, which sets in motion the so-called second-messenger mechanism of hormone action. On the other hand, steroid and thyroid hormones act predominantly by diffusing through the target cell membranes and combining with cytoplasmic or nucleic receptors to form a complex that then brings about the hormone's action.

373.

C. The adrenal medulla produces 80% epinephrine and 20% norepinephrine (noradrenalin). Metanephrine is a metabolite of epinephrine. Dopamine, a catecholamine, is a precursor of norepinephrine. Norepinephrine is converted to epinephrine by an enzyme, *N*-methyltransferase, which is present almost exclusively in the adrenal medulla. A tumor of the chromaffin tissue, called a pheochromocytoma, secretes excessive amounts of epinephrine. Ninety percent of pheochromocytomas are in the adrenal medulla. The increased levels of epinephrine from the pheochromocytoma cause hypertension. Although hypertension caused by a pheochromocytoma is rare, a correct diagnosis is very important because pheochromocytoma is one of the few causes of hypertension that is curable by surgery.

374.

A. The majority of pheochromocytomas (rare tumors) occur in the adrenal medulla, causing increased secretion of the catecholamines. As a screening test for this disorder, quantification of urinary metanephrine, the methylated product of epinephrine, is suggested because false negatives seldom occur. Follow-up testing should include measurement of urinary vanillylmandelic acid (VMA), because VMA is the primary metabolite of epinephrine and norepinephrine.

375.

B. Antidiuretic hormone (ADH), also known as vasopressin, is a peptide hormone secreted by the posterior pituitary gland under the influence of three major stimuli: decreased serum osmolality, increased blood volume, or psychogenic factors. ADH increases the renal reabsorption of water by increasing the permeability of the collecting ducts, with the result that body water is retained and urine osmolality increases. Diabetes insipidus is the syndrome that results from decreased secretion of ADH from any cause. Serum levels of ADH can be measured, but usually the measurement of serum and urine osmolality is sufficient to indicate the severity of the disease.

376.

D. Neuroblastoma is a solid malignant tumor found in the medulla of the adrenal gland, or it may arise from the extra-adrenal sympathetic chain. More commonly the disease occurs in children under the age of 5 years. Metastasis may occur to the liver, bone, bone marrow, or brain. Neuroblastoma is characterized by tumor production of epinephrine, norepinephrine, and dopamine, so all three hormones will be increased in the blood. The end product of dopamine metabolism is homovanillic acid (HVA). The end product of the catecholamines, epinephrine and norepinephrine, is vanillylmandelic acid (VMA). Both HVA and VMA will be excreted in excess in the urine.

377.

C. Serotonin (5-hydroxytryptamine or 5-HT) is synthesized from tryptophan in a variety of tissues, with the majority found in the argentaffin (enterochromaffin) cells of the intestine. Abdominal carcinoid is a metastasizing tumor of those cells and is associated with excessive production of serotonin. Serotonin in the blood is found almost exclusively in the platelets and is rapidly oxidized in the lungs to 5-hydroxyindoleacetic acid (5-HIAA), its major urinary metabolite. Urinary levels of 5-HIAA may also be increased by eating foods such as bananas and avocados, which are rich in serotonin; by the use of certain drugs such as the phenothiazines; and by carcinoid tumors.

378.

D. Somatostatin is also known as growth hormone-inhibiting hormone (GHIH). Somatostatin is a 14-amino-acid peptide that is secreted by the hypothalamus and is an inhibitor of growth hormone (somatotropin) secretion by the pituitary. It is also secreted by a variety of other organs and is a powerful inhibitor of insulin and glucagon secretion by the pancreas. Somatostatin can be measured by immunoassay methods, but its concentration in the peripheral circulation is extremely low, making it likely that its action is mostly at or near the site of secretion.

379.

B. Growth hormone (somatotropin) is a polypeptide secreted by the anterior pituitary. It is essential to the growth process of cartilage, bone, and a variety of soft tissues. It also plays an important role in lipid, carbohydrate, and protein metabolism of adults. During the growth phase of humans, hyposecretion of somatotropin results in dwarfism, whereas hypersecretion, conversely, causes pituitary gigantism. After the growth phase, hypersecretion of somatotropin causes acromegaly. Diagnosis of hypersecretion or hyposecretion of growth hormone usually requires the use of suppression or provocative tests of growth hormone release. Growth hormone levels may be quantified using immunoassay methods, including chemiluminescence immunoassay.

380.

C. Somatomedins, insulin-like growth factors I and II, is the designation given to a family of small peptides whose formation in the liver is under the control of growth hormone. The somatomedins exhibit similar activity as insulin and are active in stimulating many aspects of cell growth, particularly that of cartilage. Blood levels of somatomedin have been determined by radioimmunoassay methods, and acromegalic adults have been shown to have significantly elevated levels in comparison with normal adults.

381.

A. Calcitonin is a calcium-lowering hormone secreted by the parafollicular or C cells of the thyroid. Calcitonin acts as an antagonist to parathyroid hormone (PTH) action on the bone and kidneys. Medullary carcinoma of the thyroid is a neoplasm of the parafollicular cells that usually results in elevated serum levels of calcitonin. If the fasting calcitonin level is within the normal reference interval in a patient with suspected medullary carcinoma, a provocative calcium infusion test is often useful in improving the sensitivity of the test.

382.

A. Thyroglobulin is a glycoprotein in which the thyroid hormones are stored in the thyroid gland. When tyrosine residues of the thyroglobulin are iodinated, monoiodotyrosine (MIT) and diiodotyrosine (DIT) are formed. These iodotyrosine residues are not hormones. Triiodothyronine (T_3) and thyroxine (T_4) are the hormones produced by the thyroid, being formed by the coupling of either MIT or DIT residues. T_4 is the predominant form of the thyroid hormones secreted into the circulation, having a concentration in the plasma significantly greater than T_3. However, in terms of physiological activity, T_3 must be considered because it is four to five times more potent than T_4. Thus the overall contribution of T_3 to the total physiological effect of the thyroid hormones on the body is very significant.

383.

B. The thyroid gland is composed of two lobes connected by a structure called the isthmus. The lobes consist of many follicles. The follicle, in the shape of a sphere, is lined with a single layer of epithelial cells. The epithelial cells produce T_3 and T_4, which are stored as a component of the thyroglobulin. Within the lumen of the follicle is colloid. Thyroglobulin, secreted by the epithelial cells, makes up 90% of the colloid. As the epithelial cells synthesize the thyroid hormones, the hormones are stored in the thyroglobulin molecule. Thyroglobulin is then secreted into the colloid of the follicular lumen. When the thyroid hormones are needed, they are absorbed by the epithelial cells from their storage site, and through proteolysis, the hormones are released from fragments of the thyroglobulin molecule. T_3 and T_4 are then secreted by the cells into the blood.

384.

A. A small amount of reverse T_3 (rT_3) is made in the thyroid gland, but the majority is made from peripheral deiodination of T_4. rT_3 varies from T_3 in that rT_3 contains one iodine atom in the tyrosyl ring and two iodines in the phenolic ring, whereas T_3 has two iodines in the tyrosyl ring and one iodine in the phenolic ring. rT_3 does not have any physiological action as it is metabolically inactive. However, increased levels of rT_3 are associated with nonthyroidal illness (NTI), which also manifests with decreased levels of total T_3.

385.

C. Currently, the suggested term for autoantibodies that bind to TSH receptor sites is thyrotropin-receptor antibodies (TRAb). The thyrotropin-receptor antibodies (TRAb) are thyroid-stimulating immunoglobulins (TSI) that are IgG autoantibodies and are able to bind to the thyroid-stimulating hormone (TSH) receptor sites on thyroid cell membranes, thus preventing TSH from binding. These autoantibodies interact with the receptors similarly to TSH, thus stimulating the thyroid to secrete thyroid hormones. Because these autoantibodies do not respond to the negative feedback system as does TSH, hyperthyroidism is the end result. The majority of patients with Graves hyperthyroid disease exhibit high titers of TRAb.

386.

C. Graves disease is a name given to a diffusely hyperactive thyroid that produces thyrotoxicosis. Thyrotoxicosis results from elevated levels of thyroid hormone; therefore, laboratory results for free thyroxine (FT_4) and free triiodothyronine (FT_3) would be increased, thyroid hormone binding ratio (THBR) increased, and thyroid-stimulating hormone (TSH) decreased. In hyperthyroidism, the THBR is increased because thyroxine-binding globulin (TBG) is saturated with endogenous T_4. TSH levels are decreased because of the negative-feedback control of the thyroid hormones on the anterior pituitary.

387.

C. Hypothyroidism is a systemic disorder in which the thyroid gland does not secrete sufficient thyroid hormone. Myxedema is commonly used synonymously for hypothyroidism. Hypothyroidism can result from various diseases. If the disease affects the thyroid itself, it is referred to as primary hypothyroidism. If there is TSH deficiency of the pituitary gland, it is termed secondary hypothyroidism. Tertiary hypothyroidism is caused by hypothalamic failure that results in a decreased secretion of thyrotropin-releasing hormone. Thyroid failure in the newborn is termed cretinism. The free T_4 level and the thyroid hormone binding ratio (THBR) are decreased because of inadequate secretion of hormones. Since the thyroid hormones are low in concentration, the feedback mechanism to the anterior pituitary gland is triggered to increase production of TSH.

388.

B. To distinguish between a hypothalamic disorder and a disorder of the pituitary gland, thyroid-releasing hormone (TRH) is administered. In the case of a hypothalamic disorder (tertiary hypothyroidism), the TRH administered will cause an increased excretion of pituitary hormone, TSH. However, if the disorder originates in the pituitary gland (secondary hypothyroidism), the administration of TRH will have no effect on the pituitary gland and thus no increased excretion of TSH. Because the values of TSH were low before and remained low after administration of TRH, the disorder is secondary hypothyroidism. Primary hypothyroidism is caused by failure of the thyroid gland itself and is not evaluated by use of the TRH stimulation test. Iodine deficiency would cause high levels of TSH, and administration of TRH is not used to evaluate this disorder.

389.

B. Antibodies to thyroglobulin (TgAb) and thyroid cell peroxidase (TPOAb) are produced in several thyroid diseases. Very high antibody titers for antithyroglobulin antibodies and the detection of antithyroid peroxidase antibodies are highly suggestive of Hashimoto thyroiditis (a type of hypothyroidism). These antibodies are also frequently detected in primary myxedema and Graves disease by means of hemagglutination methods. It should be noted that antithyroid antibodies do occur in other thyroid diseases, but their prevalence is less. These antibodies have also been detected in 5–10% of the normal population.

390.

B. Almost all the triiodothyronine (T_3) and thyroxine (T_4) hormones are reversibly bound to the serum proteins, thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), and albumin. Most T_3 is bound to TBG, whereas 70% of T_4 is bound to TBG, 20% to TBPA, and 10% to albumin. T_3 has a lower affinity for TBG and TBPA than T_4. Thyroglobulin is manufactured and stored in the thyroid follicle and is not released into the circulation.

391.

C. Due to increased protein synthesis, the binding capacity of thyroxine-binding globulin (TBG) is increased in situations such as pregnancy and administration of oral contraceptives. The increased total thyroxine (total T_4) levels in these situations do not reflect the functional state of the thyroid gland. It is important when interpreting total T_4 levels to take into consideration situations such as these. Free T_4 is not affected by variations in thyroxine-binding proteins and better reflects the metabolic state that is euthyroid. However, use of the thyroid hormone binding ratio (THBR), which measures the unoccupied binding sites of TBG, in conjunction with the free and total T_4 levels permits a better interpretation of thyroid function. By this process it can be seen where the primary change occurs, whether in the level of T_4 or in TBG-binding capacity.

392.

B. In cases of peptic ulcer, treatment may include surgery that severs the vagus nerve. This severing is known as vagotomy, which, if complete, prevents the secretion of gastrin and HCl by the stomach. The Hollander insulin test is performed to assess the completeness of the vagotomy. If the vagotomy is complete, the hypoglycemia caused by the administration of insulin will not exert its normal stimulatory effect on gastric HCl and pepsinogen secretion.

393.

C. Gastrin is the designation given to a family of protein hormones produced by the mucosal cells of the gastric antrum. Once secreted, gastrin is carried in the blood to the fundic cells, causing release of hydrochloric acid. Serum gastrin levels are markedly elevated in the Zollinger-Ellison syndrome, a neoplastic proliferation of the non-beta cells of the pancreatic islets. Gastrin levels may also be elevated in pernicious anemia, duodenal ulcer disease, and gastric ulcer disease.

394.

B. Measurement of PTH during surgery for adenoma resection of the parathyroid glands assists the surgeon in determining completeness of the resection based on the rapid fall of PTH. At least three samples are needed: first, a pre-incision baseline sample as surgery starts; a second baseline sample following exposure of the gland because PTH will increase with any manipulation of the tissue; and a post-excision sample drawn 10 minutes following gland removal (some surgical protocols may require multiple sampling at 5 minutes, 10 minutes, and 20 minutes post-excision). In general, at 10 minutes post-excision, the PTH level should fall to 50% or less of the pre-incision value or the value at the time of gland resection. If the PTH value remains increased and such a decrease does not occur or if the PTH rises again after what initially appeared to be a decrease, multigland disease or ectopic production need to be investigated.

Therapeutic Drug Monitoring and Toxicology

395.

D. The term "carboxyhemoglobin saturation" refers to the fraction of circulating hemoglobin combined with carbon monoxide. Nonsmokers generally have carboxyhemoglobin saturations ranging from 0.5 to 1.5%. Fatal carbon monoxide poisoning is usually associated with carboxyhemoglobin saturations of more than 60%, and acute symptoms begin to appear at saturations of 20%. Cigarette smokers exhibit levels of 8–9% carboxyhemoglobin, but occasionally saturations of greater than 16% have been reported in heavy smokers.

396.

D. Gas-liquid chromatography (GLC) is one of the few methods that can quantify ethanol reliably in the presence of isopropanol (2-propanol) or other alcohols. Examples of the analytical problems associated with quantifying alcohols are as follows: Isopropanol significantly cross-reacts (6%) in the widely used alcohol dehydrogenase (ADH) method for ethanol; other alcohols will cross-react with dichromate methods for ethanol; and other alcohols will cross-react with the permanganate-chromotropic acid method, which is sometimes used for the identification of methanol. Because GLC is not generally available in stat laboratories, for patients with suspected exposure to alcohols other than ethanol, a variety of other laboratory and clinical findings are often used.

397.

A. A significant fraction of absorbed isopropanol is metabolized to acetone and rapidly excreted in the urine. Because of isopropanol's relatively low molecular weight, exposure to this compound will in most cases significantly increase the patient's serum osmolality. Of course, other alcohols will have a similar effect. Urine osmolality exhibits a wide variability throughout the day and, therefore, would be of little use in determining isopropanol exposure. Serum sodium would be only secondarily affected by isopropanol exposure.

398.

D. The Reinsch test is applied to urine and is based on the ability of copper to reduce most metal ions to their metallic states in the presence of acid. Cyanide is not a metal and, therefore, will not be reduced. Increased urinary levels of arsenic, bismuth, antimony, and mercury will coat the copper with dull black, shiny black, blue-black, and silver-gray deposits, respectively. The test is intended as a rapid screening method only, and results should be confirmed by more sensitive and specific methods.

399.

B. Heroin (diacetylmorphine), an abused drug, is a derivative of morphine. The morphine used in its synthesis is generally obtained from opium. Although heroin itself is not pharmacologically active, it does have a rapid onset of action. It is converted quickly to 6-acetylmorphine and then hydrolyzed to morphine, both of which are pharmacologically active. So heroin abuse can be detected by measuring its metabolite morphine in the blood or urine.

400.

B. Morphine, codeine, and heroin are collectively referred to as opiates. Codeine is found in many prescription medicines and is rapidly metabolized after absorption into morphine and norcodeine. Because blood concentrations of most opiates are low even in overdose, screening is usually done on the urine. Immunoassay or colorimetric methods can be used for screening purposes, but chromatography is generally required for quantification of specific compounds. Gas chromatography/mass spectroscopy (GC/MS) is useful for the quantification of morphine and codeine.

401.

B. THC (Δ^9-tetrahydrocannabinol) is the principal active component of marijuana. Homogeneous enzyme immunoassay methods test for the presence of THC metabolites, especially 11-nor-Δ^9-THC-9-carboxylic acid, which is the primary urinary metabolite. Metabolites appear in urine within hours of smoking and continue to be detectable for 3 to 5 days following exposure.

402.

B. Cocaine is an abused drug and not available for therapeutic use. After absorption, cocaine in the blood is rapidly converted into ecgonine and benzoylecgonine. Because of the kidney's concentrating effect, examination of the urine for the metabolites is a sensitive method of determining exposure to cocaine.

403.

C. After absorption, lead is distributed into an active pool in the blood and soft tissue and a storage pool in bone, teeth, and hair. In blood, the majority is found in erythrocytes, with only minor quantities in plasma or serum. Lead is mainly excreted by the kidney; hence urine or whole blood would be appropriate specimens for determining lead exposure. Provision for lead-free sample containers is a major requirement. Lead analysis can be done accurately by flameless atomic absorption or anodic stripping voltammetry.

404.

B. Lead interferes in heme biosynthesis at several stages, the last of these being the incorporation of iron into the tetrapyrrole ring. This alteration in biosynthesis results in the formation and accumulation of zinc protoporphyrin (ZPP), with zinc replacing the iron in the tetrapyrrole ring. Free erythrocyte protoporphyrin is the extraction product of the zinc metabolite and is a sensitive screening method for determining lead exposure above 25 μg/dL. The test is not as specific as accurate determination of lead content, however, because iron-deficiency anemia and erythropoietic protoporphyria give false-positive results. Caution must be exercised in monitoring children under 6 years of age, because the Centers for Disease Control and Prevention has defined the acceptable blood level for lead to be less than 10 μg/dL in young children. At this level, ZPP and erythrocyte protoporphyrin assays are not sufficiently sensitive.

405.

D. After absorption, mercury rapidly accumulates in many organs and in the central nervous system, with only minor quantities found in the blood. The excretion of mercury by the kidney generally forms the basis for measurement of exposure. The preferred specimen in screening for exposure to methanol or acetaminophen is serum. Whole blood is required for determining carbon monoxide exposure, because practically all the inhaled carbon monoxide is found in erythrocytes bound to hemoglobin. Following release of carbon monoxide from hemoglobin, the CO gas can be measured using gas chromatography. The percent carboxyhemoglobin saturation of whole blood can be determined by differential spectrophotometry or by using an automated, wavelength system such as a CO-oximeter.

406.

C. The term "half-life" refers to the time required for a 50% decrease in serum drug concentration after absorption and distribution are complete. The more complete descriptive term is "drug elimination half-life." It requires 5–7 half-life periods for drug concentration to reach steady state. At steady state, the drug concentration is in equilibrium with the dose administered rate and the elimination rate. Knowledge of a drug's half-life is important both for planning therapy and for monitoring drug concentration. In disease states, particularly involving the kidney and liver, half-life may be significantly altered and lead to accumulations of the drug or its metabolites in the blood.

407.

C. The Trinder reaction or modification is used almost routinely in the determination of salicylate and is based on the colorimetric reaction with ferric ions. The availability of rapid quantification in cases of salicylate overdose has been particularly useful because of the necessity of determining the drug's elimination half-life. Most clinically used thin-layer chromatographic methods are insensitive to the presence of salicylate. Because the colorimetric reaction used for determining the presence of phenothiazines with ferric perchloric-nitric (FPN) reagent is dependent on ferric ions also, false-positive reactions in the ferric ion methods for salicylate may be expected.

408.

D. Hepatotoxicity is common in acetaminophen overdose. It is particularly important to be able to determine the acetaminophen serum level rapidly so that the elimination half-life of the drug can be estimated. Hepatic necrosis is more common when the half-life exceeds 4 hours and is very likely when it exceeds 12 hours. The concentration of acetaminophen can be measured by HPLC, colorimetric, EMIT, and fluorescence polarization methods.

409.

A. The barbiturates are classified pharmacologically according to their duration of action. Phenobarbital is long acting, amobarbital and butabarbital are intermediate acting, and pentobarbital and secobarbital are short acting. In general, the long-acting barbiturates have higher therapeutic and toxic levels than the shorter-acting barbiturates. In cases of overdose, it is important to be able to identify the type of barbiturate in the blood for correct therapy. Measurement of specific barbiturates usually requires chromatography or immunoassay.

410.

B. Tobramycin and gentamicin are examples of aminoglycoside antibiotics. Their use has been associated with both nephrotoxicity and ototoxicity. Drug concentration monitoring of patients taking the aminoglycosides requires an analytic system with good precision and accuracy over a wide range because both peak and trough levels are usually monitored. The trough level is used mainly as a measure of nephrotoxicity, whereas the peak level is useful in determining whether adequate therapy is being given to eliminate the causative organism.

411.

C. Although digoxin, nortriptyline, and quinidine have various effects on cardiac arrhythmias, they do not have metabolites with similar activity. Procainamide is an antiarrhythmic drug and has at least one metabolite with the same activity, namely, *N*-acetylprocainamide (NAPA). Because of differences in half-life, NAPA may accumulate in the blood and produce toxic effects even with therapeutic levels of procainamide. Therefore, both procainamide and NAPA need to be quantified for therapeutic drug monitoring.

412.

A. Drugs in the free state are able to elicit a pharmacologic response. It is the free drug that is able to cross cell membranes and to bind at receptor sites. In the protein-bound state, drugs are unable to enter tissues and interact at receptor sites.

413.

B. Acute glomerulonephritis is characterized by hematuria and albuminuria. The hypoalbuminemia results in less protein-bound drug and an increase in free drug. Thus, more free drug is available in the circulation to enter the tissues. Such a situation may result in severe side effects and even toxic effects. Therefore, to properly regulate drug dosages, it is advisable to measure free drug levels in blood, rather than total drug levels, whenever possible.

414.

B. The term "free drug" refers to the fraction of drug in the plasma not bound to protein. For the determination of free drug concentrations, urine would not be the proper specimen because the rate of drug excretion depends mainly on conjugation or metabolism and not on protein binding. In preparation of a protein-free filtrate of plasma, the drugs bound to protein would also enter the filtrate because they are dissociated when the protein is denatured. Saliva is a form of plasma ultrafiltrate and with some restrictions as to sampling and type of drug analyzed can be used for free-drug monitoring. Methods for equilibrium dialysis and for preparation of ultrafiltrates of plasma are now available and can provide excellent samples for free-drug analyses of some compounds.

415.

C. Tacrolimus (Prograf) is an antibiotic that functions as an immunosuppressant in organ transplantation, especially in liver transplants. By inhibiting interleukin production, it blocks lymphocyte proliferation. Adverse reactions to the drug include nephrotoxicity, nausea, vomiting, and headaches. Other immunosuppressant drugs include cyclosporine, mycophenolic acid, and sirolimus. Methotrexate is an antineoplastic drug, amiodarone is an antiarrhythmic drug, and paroxetine is an antidepressant drug.

416.

B. Theophylline, a xanthine with bronchodilator activity, is widely used in the treatment of asthma. Because of its availability and potential toxicity, it can also be subject to accidental overdose. Chromatographic methods are effective in separating theophylline from caffeine and theobromine, which are two commonly occurring and potentially interfering xanthines. However, most clinical thin-layer chromatographic methods are relatively insensitive to the xanthines, and suspected theophylline overdose should be confirmed by HPLC or immunoassay methods.

417.

D. Following absorption, primidone is metabolized primarily to phenobarbital and secondarily to phenylethylmalonamide (PEMA). Both metabolites have anticonvulsant activity, and both have a longer half-life than primidone. Generally, only serum phenobarbital and primidone concentrations are monitored. Determination of phenobarbital is particularly important when another anticonvulsant phenytoin is also administered because the metabolic rate of primidone conversion to phenobarbital may be increased, with a resulting accumulation of phenobarbital in the blood.

418.

A. Amitriptyline, doxepin, and imipramine and their active metabolites nortriptyline, nordoxepin, and desipramine, respectively, are tricyclic compounds particularly useful in the treatment of endogenous depression. These compounds are lipid soluble and, therefore, highly protein bound in the plasma. Although toxic concentrations of these drugs often lead to cardiac arrhythmias, low concentrations have been found to have antiarrhythmic activity. Because of these varying biological effects at differing serum concentrations, there is a need both for monitoring in cases of therapy and screening for toxic effects in cases of overdose.

419.

B. Lithium is used in the treatment of manic depression. Because of the small difference between therapeutic and toxic levels in the serum, accurate measurements of lithium concentrations are essential. It is also important to standardize the sample drawing time in relation to the previous dose. Measurement is made by ion-selective electrode electrochemical analysis or by atomic absorption spectrophotometric analysis.

420.

C. The collection of blood samples for therapeutic drug monitoring requires both the selection of the proper time for sampling and the recording of that time on the report. It is essential that the drug level be related in time to the time of the previous and/or the next drug administration. Collection of blood samples is generally avoided during the drug's absorption and distribution phases. When peak levels of the drug are required, the blood sample must be drawn at a specified time after drug administration. Trough levels are most reliably determined by collecting the blood sample before the next drug administration.

421.

C. Persons involved in therapeutic drug monitoring should consider not only the properties of the various drugs but also the populations to which they are administered. The neonate is particularly susceptible to drug toxicity because of renal and hepatic immaturity, which leads to an increased drug half-life in comparison with that seen in adults. The neonatal pattern of drug elimination is reversed rapidly several weeks after birth, and children generally metabolize drugs more rapidly than adults. With the onset of puberty, the rate of drug metabolism generally slows and approaches the adult rate of drug use.

422.

C. Within the systemic circulation a drug will either remain free or will bind to protein. Generally, acidic drugs bind to albumin, and basic drugs bind to such globulins as alpha$_1$-acid glycoprotein (AAG). Occasionally, a particular drug may bind to both types of protein.

423.

D. Chlorpromazine (Thorazine®) and thioridazine are examples of phenothiazines and are used in the treatment of psychoses. Although the drugs themselves have a relatively short half-life, metabolites may be found in the urine for many weeks after cessation of therapy. Screening for phenothiazines is often done by specific chromatographic techniques or by the less specific ferric perchloricnitric (FPN) colorimetric reagent. Quantification is done by HPLC and fluorescent polarization immunoassay (FPIA).

424.

A. Phenytoin is the recommended name for the anticonvulsant diphenylhydantoin. Because of its wide use and toxicity at high concentrations, phenytoin is often the subject of overdose. Thin-layer chromatography or spectrophotometry is used for screening. Quantification usually requires gas- or high-performance liquid chromatography or immunoassay (e.g., EMIT, FPIA).

425.

D. Diazepam (Valium®) is an example of a benzodiazepine. This group of drugs is used for the treatment of anxiety. Oxazepam is an active metabolite of diazepam and is also available as a prescribed drug (Serax®). Detection of oxazepam glucuronide in the urine is used as a screening method for diazepam. Quantification of the benzodiazepines may be achieved using HPLC.

426.

D. The major active metabolite of procainamide is *N*-acetylprocainamide (NAPA). Procainamide is an antiarrhythmic drug that is used to treat such disorders as premature ventricular contractions, ventricular tachycardia, and atrial fibrillation. Because procainamide and its metabolite NAPA exhibit similar and cumulative effects, it is necessary that both be quantified to assess therapy. Methods for their analysis include GC, HPLC, FPIA, and EMIT.

427.

A. Theophylline is a bronchodilator that is used to treat asthma. The therapeutic range is 10–20 μg/mL, and use must be monitored to avoid toxicity. Use of theophylline has been replaced where possible with β-adrenergic agonists, which are available in the inhaled form.

Vitamins

428.

A. HPLC is a commonly used technique for the measurement of vitamins. Measurement by HPLC tends to be rapid, sensitive, and specific. Other techniques employed include spectrophotometric, fluorometric, and microbiological assays.

429.

B. Ascorbic acid is commonly known as vitamin C. Because humans are unable to synthesize ascorbic acid, it is necessary that it be taken in through the diet. If ascorbic acid is not ingested in a sufficient amount, a deficiency develops that leads to the disease known as scurvy. Scurvy is characterized by bleeding gums, loose teeth, and poor wound healing.

430.

B. The term "lipid" encompasses a large group of compounds, including the sterols, fatty acids, triglycerides, phosphatides, bile pigments, waxes, and fat-soluble vitamins. Vitamins A, D, E, and K are classified as fat-soluble vitamins. Thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), cyanocobalamin (B_{12}), niacin, pantothenic acid, lipoic acid, folic acid, inositol, and ascorbic acid (C) are classified as water-soluble vitamins and as such are not lipid compounds.

431.

C. The definitive test for the diagnosis of steatorrhea (fat malabsorption) is the fecal fat determination that usually is done with a 72-hour collection. Carotenoids are a group of fat-soluble compounds that are precursors of vitamin A (retinol). The carotenoids are not synthesized in humans, and their absorption depends on intestinal fat absorption. Therefore, the serum carotene level is sometimes used as a simple screening test for steatorrhea. In addition to steatorrhea, other conditions, such as poor diet, liver disease, and high fever, can result in below-normal carotene levels. Folic acid and vitamins C and B_{12} are water soluble and would not be useful for determining fat absorption.

432.

C. Vitamin B_{12} (cyanocobalamin) is a cobalt-containing vitamin that is necessary for normal erythropoiesis. Intrinsic factor is a gastric protein that specifically binds vitamin B_{12} and carries it to the ileum for absorption. The transcobalamins are a group of plasma proteins, some of which bind vitamin B_{12} and some of which bind both vitamin B_{12} and cobalamin analogs. The cobalophilins (R proteins) are those transcobalamins that can also bind the cobalamin analogs.

433.

A. Vitamin B_{12} is a water-soluble vitamin. It is absorbed in the gastrointestinal tract by way of a substance called intrinsic factor. Deficiency of vitamin B_{12} produces a megaloblastic anemia. Anemia caused by a deficiency of vitamin B_{12} because of a lack of intrinsic factor (IF) is called pernicious anemia. The Schilling test (with and without IF) is used to diagnose pernicious anemia. It is helpful in distinguishing pernicious anemia from other malabsorption syndromes. A positive Schilling test indicates low absorption of B_{12} without IF and normal absorption with IF. However, in diseases of the small bowel, low absorption occurs with and without IF.

434.

A. The designation "vitamin D" applies to a family of essential fat-soluble sterols that includes vitamin D_3 or cholecalciferol. This compound can either be absorbed directly or synthesized in the skin from 7-dehydrocholesterol with the help of ultraviolet irradiation. For physiological functioning, vitamin D_3 must be metabolized first by the liver to 25-hydroxyvitamin D_3 and then by the kidney to the final hormonal product, 1,25-dihydroxyvitamin D_3 (calcitriol). The kidney also synthesizes 24,25-dihydroxyvitamin D_3 by an alternate pathway. This compound does not have the hormonal activity of calcitriol, but because of its similar structure and relatively high concentration in the serum, it has complicated the determination of serum calcitriol.

435.

D. Adequate amounts of vitamin K are required for the synthesis of prothrombin by the liver. Because prothrombin is an essential component of the clotting system, a deficiency of vitamin K leads to a deficiency of prothrombin, which results in a delayed clot formation. Several closely related compounds having vitamin K properties include phylloquinones, which are synthesized in plants, and menaquinones, which are synthesized by bacteria. Because the intestinal flora may not be developed sufficiently in the newborn, vitamin K (menaquinone) deficiency can occur. This leads to increased clotting time, which may result in hemorrhagic disease in infancy.

436.

B. Riboflavin (vitamin B_2) is a constituent of two redox coenzymes, flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD). These coenzymes, in combination with appropriate proteins, form the flavoprotein enzymes, which participate in tissue respiration as components of the electron-transport system. The property that enables them to participate in electron-transport is their ability to exist in the half-reduced form (FADH) and in the fully reduced form ($FADH_2$).

437.

A. A deficiency in thiamin (vitamin B_1) is associated with beriberi and Wernicke-Korsakoff syndrome. In general, thiamin deficiency affects the nervous and cardiovascular systems. Thiamin deficiency is sometimes seen in chronic alcoholics and in the elderly.

REFERENCES

Bishop, M. L., Fody, E. P., and Schoeff, L. (Eds.) (2005). *Clinical Chemistry Principles, Procedures, Correlations,* 5th ed. Philadelphia: Lippincott Williams & Wilkins.

Burtis, C. A., Ashwood, E. R., and Bruns, D. E. (Eds.) (2008). *Tietz Fundamentals of Clinical Chemistry,* 6th ed. Philadelphia: Saunders.

Haven, M. C., Tetrault, G. A., and Schenken, J. R. (Eds.) (1995). *Laboratory Instrumentation,* 4th ed. New York: Van Nostrand Reinhold.

Kaplan, L. A., Pesce, A. J., and Kazmierczak, S. C. (Eds.) (2003). *Clinical Chemistry Theory, Analysis, Correlation,* 4th ed. St. Louis: Mosby.

Westgard, J. O., Quam, E., and Barry, T. (Eds.) (1998). *Basic QC Practices*. Madison, WI: WesTgard® Quality Corporation.